Algal and protozoan response to Holocene climate change and anthropogenic impact: a case study from Sluice Pond, MA

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Abstract

Sluice Pond is a small (18 ha) and deep (*Zmax* 20.0 m) partially meromictic, pond in Lynn, Massachusetts that contains a diverse dinocyst record since the early Holocene. High dinocyst concentrations, including morphotypes not previously described, as well as the preservation of several specimens of cellulosic thecae are attributed to low dissolved oxygen (DO) in the basin. The fossil protozoan record supports the interpretation- thecamoebians were unable to colonize the basin until the middle Holocene and only became abundant when the drought-induced lowstand oxygenated the bottom waters. Protozoans tolerant of low DO became abundant through the late Holocene as water levels rose and cultural eutrophication produced a sharp increase in biochemical oxygen demand (BOD) beginning in the 17th century. Recent sediments contain a dominance of *Peridinium willei*, indicating cultural eutrophication and the planktonic ciliate *Codonella cratera* and the thecamoebian *Cucurbitella tricuspis* in the deep basin. Above the chemocline however, a diverse difflugiid thecamoebian assemblage is present.

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List of Abbreviations

%A	percent relative abundance
AD	anno domini
A(h)	area in hectares
As	arsenic
BOD	biochemical oxygen demand
^{14}C	radiocarbon
Ca	calcium
cal yr BP	calibrated radiocarbon date
Cd	cadmium
Cl	chlorine
CLAM	‘classic’ age-depth modelling
cm	centimetre
Co	cobalt
Cu	copper
Cr	chromium
DBD	dry bulk density
DO	dissolved oxygen
DOC	dissolved organic carbon
E	error in percent
F	relative abundance
g	gram
GPS	global positioning system
GTA	Greater Toronto Area
ha	hectare
HCl	hydrochloric acid
HF	hydrofluoric acid

Hg	mercury
ka	kiloannus
LEL	low effect level
LIS	Laurentide ice sheet
LOI	loss on ignition
LSU rDNA	large subunit deoxyribonucleic acid
m	metre
m bmll	metres below modern lake level
m^2	metre squared
mg/kg	milligrams per kilogram
ml	millilitre
MOE	Ministry of the Environment
N	nitrogen
N	north
N	number of specimens
N%	total nitrogen
NAP	non-arboreal pollen
NEL	no effect level
Ni	nickel
NPP	non-pollen palynomorph
OC	organic carbon
OC/N	organic carbon-nitrogen ratio
OC/S	organic carbon-sulphur ration
P	phosphorus
Pb	lead
PCA	principal component analysis
pH	measure of the –log (H+)

ppm	parts per million
PVC	polyvinyl chloride
R^2	coefficient of determination
SA	surface area
S%	total sulphur
SDI	Shannon-Weaver diversity index
SEL	severe effect level
SEM	scanning electron microscope
SONAR	sound navigation and ranging
SP	Sluice Pond
SSU rDNA	small subunit deoxyribonucleic acid
TP	total phosphorus
W	west
WPM	woody particulate material
Zm	maximum depth
Zmax	maximum depth
Zn	zinc
Zr	relative depth
µg	microgram
$\delta^{13}C$	stable carbon isotopic composition
$\delta^{15}N$	stable nitrogen isotopic composition
$\delta^{34}S$	stable sulfur isotopic composition
π	pi

Chapter 1

1.1 Cultural eutrophication in lakes

Eutrophication, is one of the most common water-quality problems in the world (Schindler 2006). Phosphorus (P) and nitrogen (N) are nutrients that can be introduced naturally into the environment, with phosphorus being the more important limiting nutrient in freshwater environments (Schindler 1974). Cultural eutrophication, the process by which excess nutrients from anthropogenic activity cause enrichment in aquatic ecosystems, has been greatly debated since the 1960s when many lakes, rivers and reservoirs were exhibiting signs of eutrophication (Smol 2008). Anthropogenic sources of phosphorus include point sources such as untreated sewage, other municipal and industrial effluents and non-point sources like fertilizer from agricultural practices and recreational activities. Schindler (1974) and colleagues working in Lake 226 at the Experimental Lakes in northwest Ontario determined that substantial algal blooms occurred in response to the addition of phosphorus (Figure 1.1).

Figure 1.1: Lake 226, demonstrating the vital role of phosphorus in eutrophication. The lower basin, fertilized with phosphorus, nitrogen, and carbon, was covered by an algal bloom within 2 months. No increases in algae or species changes were observed in the upper basin, which was fertilized with nitrogen and carbon (from Schindler 1974).

Continued research on Lake 304 noted the marked increase of chlorophyll *a* following the introduction of phosphorus in 1971 and 1972 during the late summer (Figure 1.2). Additions of carbon and nitrogen in 1973 proved that it was primarily phosphorus that caused chlorophyll *a*

to increase in the summers of 1971-1972 (Schindler 1974). This excessive algal productivity causes dissolved oxygen (DO) levels to decrease, imparts a foul odour to the water, and restricts sunlight. The decrease in DO is caused by biochemical oxygen demand (BOD) resulting from microbial decay and when phytoplankton use more oxygen than is produced by photosynthesis (Diersing 2009). Once algal blooms become frequent in an ecosystem, cyanobacteria such as *Microcystis* can produce toxins that render water consumption unsafe. A case from Baldur, Manitoba in 1996 reported the death of 16 cows due to drinking water enriched with nerve toxins produced by cyanobacteria (Smol 2008).

Most cultural eutrophication in North American lakes can be attributed to European settlers, coinciding with the ragweed rise in pollen records (e.g. Shuman et al. 2001; Parshall and Foster 2002; Danesh et al. 2013; Paquette and Gajewski 2013; Volik 2014), but First Nations people also adversely impacted lacustrine ecosystems (Burden et al. 1986; Ekdahl et al. 2007; McCarthy et al. 2011; Krueger 2012; McCarthy and Krueger 2013).

Understanding the biological response to nutrient changes is critical in the sustainable management of freshwater ecosystems (Quinlan et al. 2008). Paleontolological records provide a multi-proxy approach to understanding past and future environments (e.g. using diatoms, chironomids), however non-pollen palynomorphs have been under-utilized for this purpose. The usefulness of palynomorphs is outlined by several factors (Komarek and Jankovska 2001; Volik 2014):

1. Their resistance to chemical treatment, such as hydrofluoric acid (HF) and hydrochloric acid (HCl);
2. Their abundance in lakes and wetlands;
3. Their global distribution.

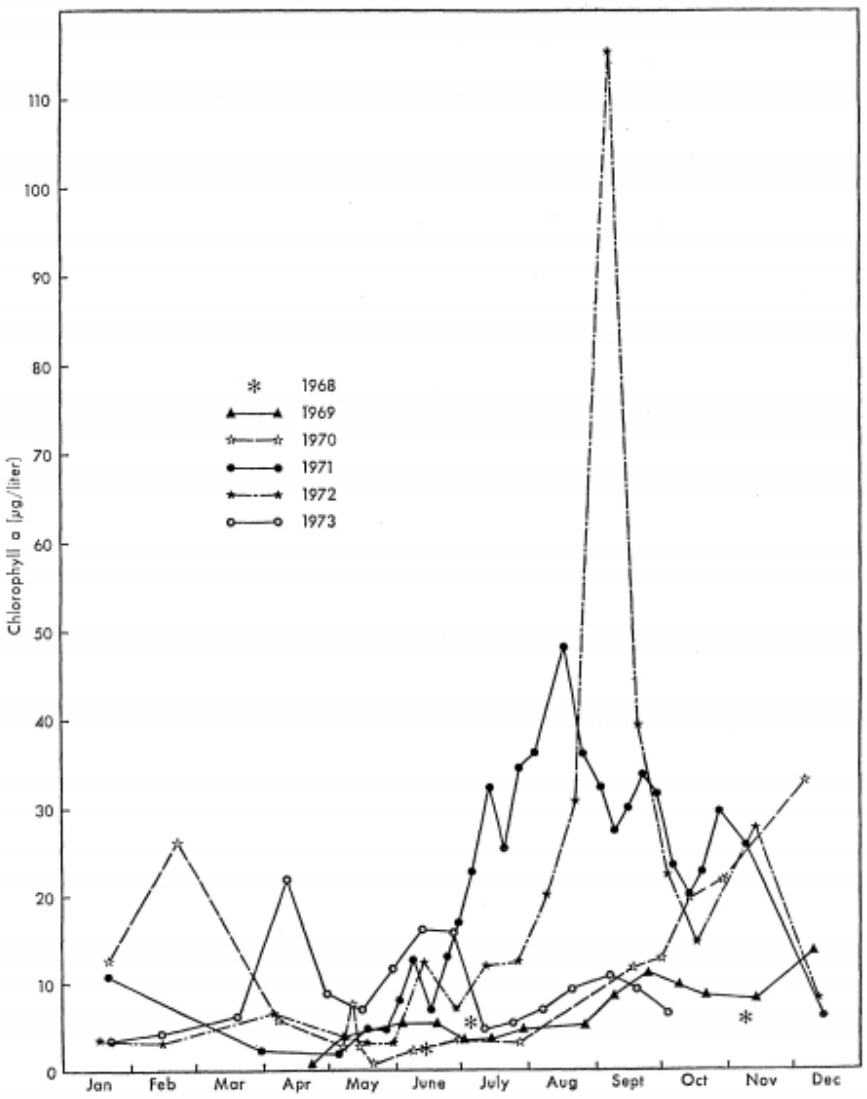

Figure 1.2: Chlorophyll *a* concentrations in Lake 304. In 1968, 1969, and 1970 the lake was not fertilized. In 1971 and 1972, it received annual additions of 0.40g of phosphorus, 5.2g of nitrogen, and 5.5g of carbon per square metre. In 1973, additions of nitrogen and carbon were continued at the same rate but phosphorus was not added (from Schindler 1974).

1.2 Heavy metal pollution in aquatic environments

Heavy metals in aquatic sediments can have adverse effects on organisms living in that environment (e.g. Buccolieri et al. 2006; Bing et al. 2012). Buccolieri et al. (2006) determined that heavy metals do not biodegrade and thus become persistent problems in aquatic ecosystems. Heavy metals such as arsenic (As) can be taken up by plants through roots or leaves and in animals through inhalation, ingestion, or permeation of the skin or mucous membranes

(Patterson et al. 1996). The lowest effect level to severe effect level for heavy metals was established by the Ministry of the Environment (2008) (Table 1.1).

	No Effect Level	Lowest Effect Level	Severe Effect Level
METALS			
Arsenic	-[b]	6	33
Cadmium	-	0.6	10
Chromium	-	26	110
Copper	-	16	110
Iron (%)	-	2	4
Lead	-	31	250
Manganese	-	460	1100
Mercury	-	0.2	2
Nickel	-	16	75
Zinc	-	120	820
NUTRIENTS			
TOC(%)[c]	-	1	10
TKN[c]	-	550	4800
TP[c]	-	600	2000

[a.] Values in µg/g dry weight unless otherwise noted (µg/g = ppm). Values less than 10 have been rounded to one significant digit. Values greater than 10 have been rounded to two significant digits except for round numbers which remain unchanged (e.g., 400).

[b.] "-" denotes insufficient data/no suitable method

[c.] TOC – Total Organic Carbon; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus

Table 1.1: Provincial sediment quality guidelines for metals and nutrients for the province of Ontario (from MOE 2008).

Elemental mercury (Hg) is rarely found in freshwater sediments except for areas where gold mining was common (Smol 2008). Methylmercury is the more common form of Hg and is found when bacteria transform mercury into its organic and more toxic bioavailable counterpart. Methylmercury is magnified in higher trophic levels (Smol 2008), therefore, organisms such as fish are greatly affected by the accumulation of Hg.

Lead (Pb) was commonly used in many everyday items such as paint, piping, automobiles, gasoline, tableware and glassware (CDC 1991). By 1992, the addition of lead to household products was halted (CDC 1991). The bioaccumulation of Pb along with Cd can lower protozoan density, thus inhibiting the base of the food chain (Fernandez-Leborans and Herrero 2000). Volcanic activity, the steel industry, zinc mining and waste incineration account for the

greatest production of cadmium (Hutton 1983). Excessive Cd accumulation in higher trophic states leads to hyperactivity, reduced growth and reproductive failure (USGS 2010).

1.3 Introduction to palynology

Palynology in Greek refers to "the study of dust", but particularly the study of microfossils of acid-resistant complex organic material such as chitin, sporopollenin, and dinosporin that range in size from 5 to 500μm. Palynomorphs include particles produced by organisms from every kingdom of life including Animalia, Plantae, Protista, Fungi, and Bacteria.

1.3.1 Pollen and spores

Pollen is produced by all seed-producing plants (Jarzen and Nichols 1996). Pollen grains are the male gametophytes of seed plants whose exine is composed of resistant polymers known as sporopollenin (MacDonald 1988). Pollen analysis has long been used as a proxy for dating climatic changes, reconstructing vegetation and determining the presence or absence of human settlement in an area (McAndrews and Boyko-Diakonow 1987). A pollen zonation developed by McAndrews (1981) outlines plant succession in southern Ontario from the Late Pleistocene (zone 1) through the Holocene (zones 2 - 4). Pollen zone 1, the *Picea* (spruce) zone, is subdivided into subzone 1a where pollen of the sedge family is high and subzone 1b where there is high spruce with low herbs and represents spruce-dominated boreal woodland (10,500 - 12,000 yr BP) (McAndrews 1981, 1994) (Figure 1.3). Zone 2 (8,200 - 10,500 yr BP), the *Pinus* (pine) zone, is dominated by pine and contains two subzones, an early 2a *Pinus banksiana/resinosa* (jack/red pine) zone and the latter subzone 2b *Pinus strobus* (white pine). Zone 3 (300 - 8,200 yr BP) is characterized as the *Fagus* (beech) zone (McAndrews 1981, 1994). Subzone 3b (3,500 - 4,700 yr BP), where *Tsuga* (hemlock) is low, separates subzones 3a and 3c. Hemlock prefers cool and wet conditions and is typically found growing on the north-facing slope of mountains where it is cooler and more moist. An increase in white pine characterizes pollen zone 3d. The decline in hemlock (zone 3b) in the mid-latitudes of North America is associated with drought (Webb 1988; Newby et al. 2000) rather than pathogens (Bhiry and Filion 1996). Zone 4 is characterized by an increase in *Ambrosia* (ragweed) pollen and other non-arboreal pollen (NAP) due to human settlement (McAndrews 1994). With the exception of zone 4, the different pollen zones represent climatic shifts since deglaciation.

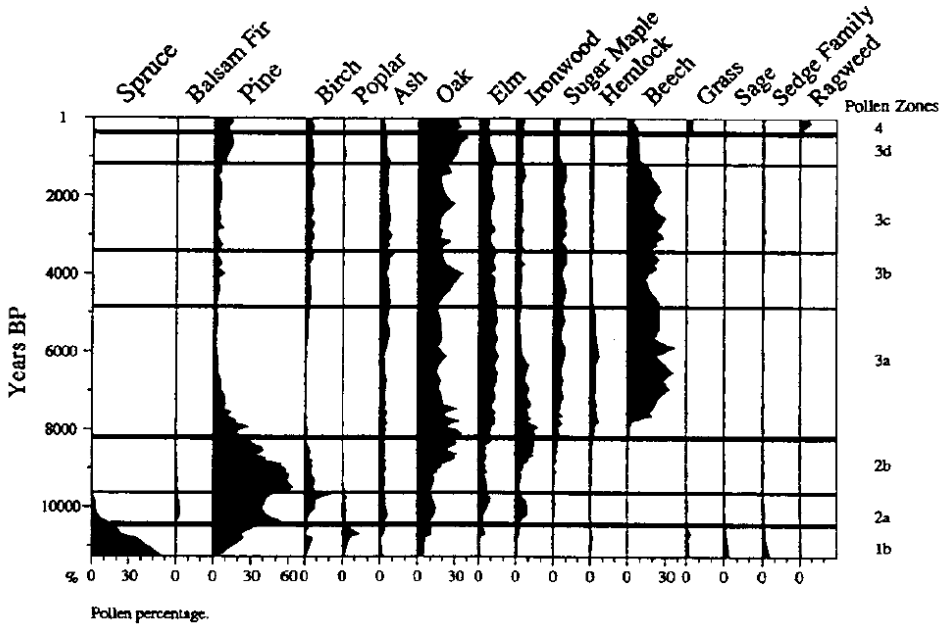

Figure 1.3: Pollen succession from Hams Lake (McAndrews 1981; Bennett 1987) using pollen succession zonation. Note the absence of pollen zone 1a where spruce pollen is high and herbs dominate (from McAndrews 1994).

A similar pollen succession records vegetation change since deglaciation in New England (Shuman et al. 2001). Massachusetts however, exhibited a shift in pollen succession earlier due to its warmer setting (Figure 1.4). Cores taken from Makepeace Swamp (Newby et al. 1999) and Crooked Pond (Shuman et al. 2001) show that: 1) white pine grew in abundance in southern New England and indicates that regional conditions were drier than today between 11,200 and 9,500 cal yr BP, 2) between 8,000 and 5,500 cal yr BP precipitation increased, 3) and between 5,500 and 3,300 cal yr BP drier conditions associated with the decline in hemlock pollen resulted in lower lake levels regionally.

In New England studies, illustrated by Crooked Pond (CP) in Figure 1.4, zone 1a is characterized by the initial decline in *Picea* and zone 1b is characterized by a decrease in *Pinus* and increase in *Picea* pollen. This second increase in *Picea* is typical of late-glacial pollen successions in New England (Deevey 1939; Leopold 1956; Davis 1969; Gaudreau and Webb 1985; Shuman et al. 2001). Zone 2a is characterized by the highest percentage of *Pinus.* In comparison to zone 2a, zone 2b contains *Pinus, Quercus, Betula* and *Tsuga*. Zone 3a is marked by increases in *Tsuga* and *Fagus*. The onset of zone 3b is characterized by a decline in *Tsuga* and

a rise in *Carya* and *Betula.* The rise in *Ambrosia* marks the base of pollen zone 4 (300 - 400 cal yr BP) (Figure 1.4) (Shuman et al. 2001)

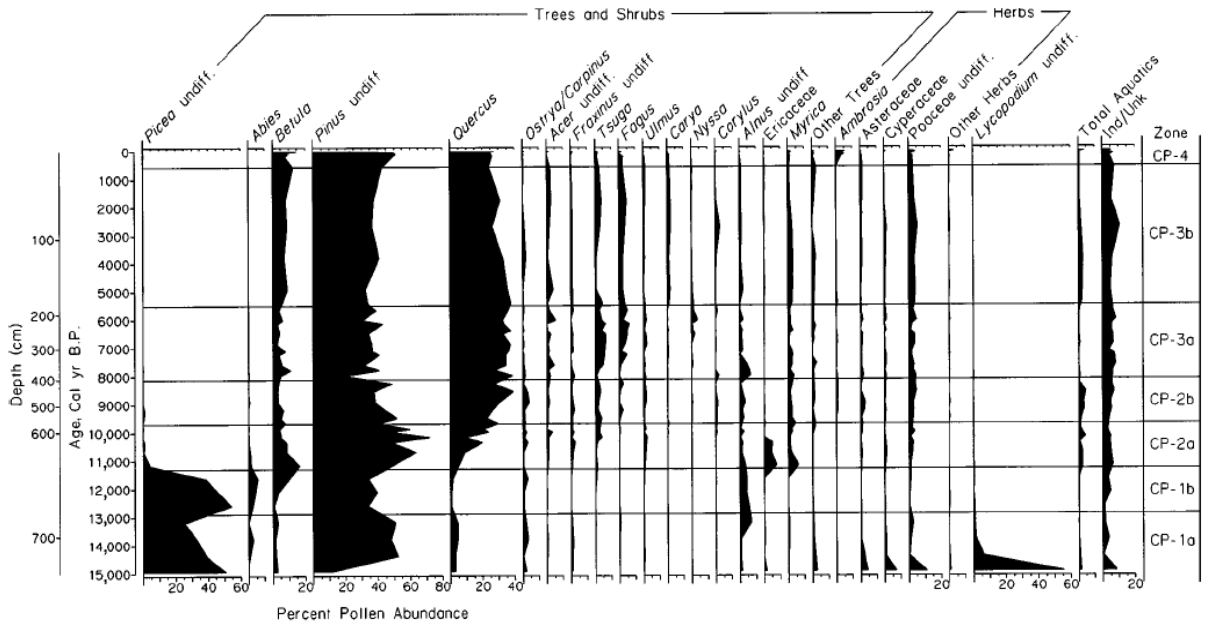

Figure 1.4: Percentage pollen diagram from Crooked Pond, MA illustrating pollen succession from the early Holocene (zone CP-1a, dominated by *Picea*) to the gradual shift to a deciduous forest (from Shuman et al. 2001).

1.3.2 An introduction to freshwater dinoflagellates

Dinoflagellates are microscopic, unicellular protists that form the base of the food chain in aquatic ecosystems. Dinoflagellates typically occur as motile cells with two flagella. The transverse flagellum encircles the dinoflagellate around a cingulum (or girdle) while the longitudinal flagellum is a whip-like "tail" that allows the dinoflagellate to rotate forward (Figure 1.5) (Fensome et al. 1996). Due to this unique rotating motion, "Dinoflagellata" comes from the Greek *dinos,* "whirling rotation" and the Latin *flagellum,* "small whip".

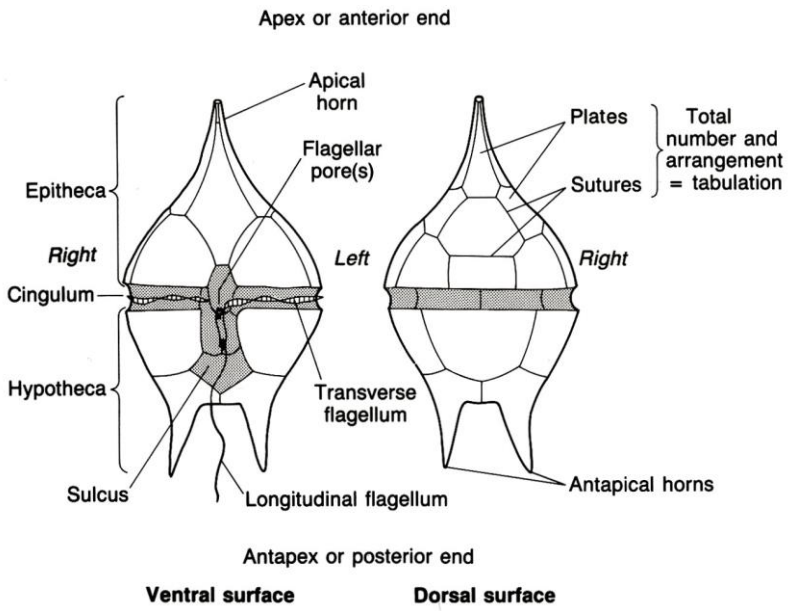

Figure 1.5: The general morphology of a dinoflagellate. Note the presence of thecal plates and flagella, used in the motile stage for swimming. These thecal plates are often reflected on the cyst itself, allowing researchers to link a motile dinoflagellate with its resting cyst (from Fensome et al. 1996).

Tabulation, the arrangement of thecal plates in the motile stage, is vital in understanding dinoflagellate morphology. These thecal plates adjoin one another along linear plate sutures, which overlap the adjacent plate. These plates are thought to be made of cellulose and as the cell grows, growth bands (also known as intercalary bands) appear (Fensome et al. 1996). The arrangement of the thecal plates is vital in understanding the cyst-theca relationship as tabulation may be imprinted on the cyst itself (Figure 1.5).

Dinoflagellates are primary producers that reach optimal growth during high illumination, temperature and nutrient levels (Taylor 1987). They are often found in the presence of decaying vegetation, as it allows for maximum uptake of nutrients (Ngo 1990). The most common genus in freshwater environments is *Peridinium* Ehrenberg, ranging in size from 10 - 100 μm in length, whereas small (20 - 25 μm x 20 - 30 μm) dinoflagellates of the Umbonatum group are assigned to the genus *Parvodinium* Carty (Carty 2008).

1.3.2.1. Systematic taxonomy for select freshwater dinoflagellates

Superkingdom Eukaryota

Kingdom Chromalveolata

Superphylum	Aveolata Cavalier-Smith 1991
Phylum	Dinoflagellate Butschli 1885
Class	Dinophyceae Pascher 1914
Order	Peridiniales Haeckel 1894
Family	Peridiaceae Ehrenberg 1831
Genus	*Chimonodinium* Craveiro et al. 2011
Genus	*Parvodinium* Carty 2008
Species	*P. inconspicuum* (Lemmerman 1899) Carty 2008
	P. umbonatum (Stein 1883) Carty 2008
Genus	*Peridinium* Ehrenberg 1832
Species	*P. gatunense* Nygaard 1925
	P. limbatum (Stokes) Lemmerman 1900
	P. volzii Lemmerman 1904
	P. willei Huitfeld-Kaas 1900
	P. wisconsinense Eddy 1930

1.3.2.2 The life cycle

When conditions become unfavourable, dinoflagellates slough off their thecae and produce a cyst in order to hibernate (Figure 1.6). For example, in the summer months, the dinoflagellate-*Peridinium volzii* Lemmerman is planktonic with a motile thecate stage. In the fall, when temperatures and light declines, a cyst begins to form, and the theca decays. In the winter months, the dinoflagellate cyst rests until conditions become favourable again (Wall and Dale 1968). Krueger (2012) germinated dinoflagellates from meromictic Crawford Lake that encysted approximately 150 years ago. These cysts are often found in palynological studies. Thecae, however, are rare as they are not composed of acid-resistant dinosporin and are prone to degradation.

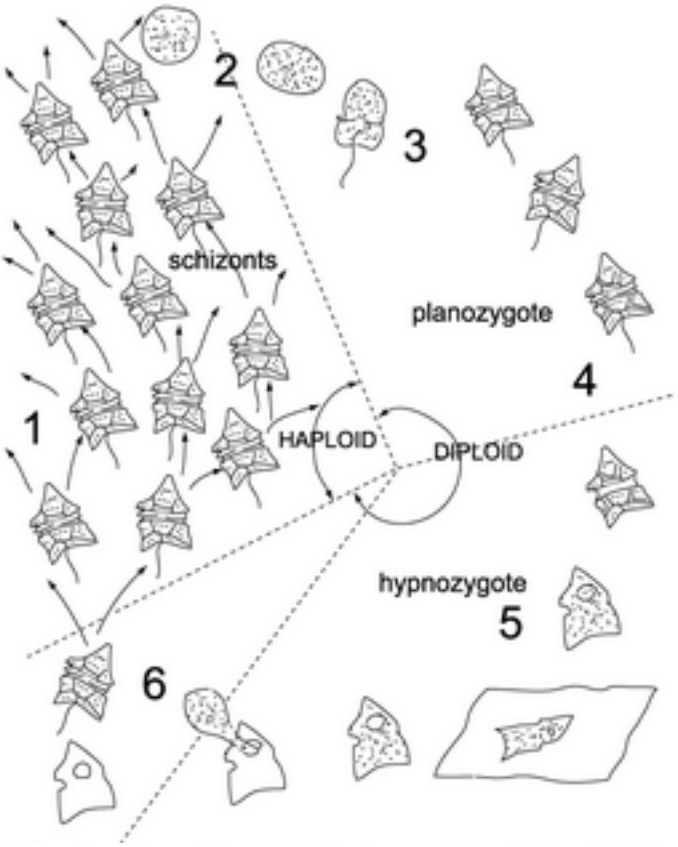

Figure 1.6: Growth and population expansion of dinoflagellates typically occurs in the spring/summer when light and temperature is optimal 1) Population expansion by vegetative division 2) Zygotes are formed where one or more theca may be lost 3) A new theca is formed 4) Cell activity progressively diminishes and a cyst forms in the theca in the fall when conditions become unfavourable (e.g. light diminishes) 5) The cyst acts as a acid-resistant sedimentary particle 6) Following dormancy, the cell excysts from the cyst (when conditions become favourable again), grows a theca and becomes motile (from Fensome et al. 1996)

Dale (1983), Pfiester and Anderson (1987), Taylor 1990, and Mertens et al. (2012) describe the term “cyst” as:

1) A pellicle cyst (or temporary cyst); which is often formed at different stages of life, often as a product of asexual reproduction. These cysts are single layered, lack ornamentation and a means of storage. These cysts are often confused with resting cysts.
2) A resting cyst (or dormant zygote); which is formed usually following sexual reproduction. These cysts remain dormant until the following growing season and may or may not germinate in the following season. These cysts are most important to

palynologists as they may fossilize. In contrast to pellicle cysts, these cysts often consist of several layers of acid-resistant dinosporin and often display paratabulation.

3) A coccoid stage; where photosynthesis is functioning in the cell.

1.3.2.3 Freshwater dinoflagellate studies

Dinocyst germination varies based on light, temperature, pH and DO. To reduce competition, some dinoflagellates bloom in different seasons. For example, sub-tropical *Peridinium gatunense* germinates in the winter (Figure 1.7) (Pollinger and Hickel 1991).

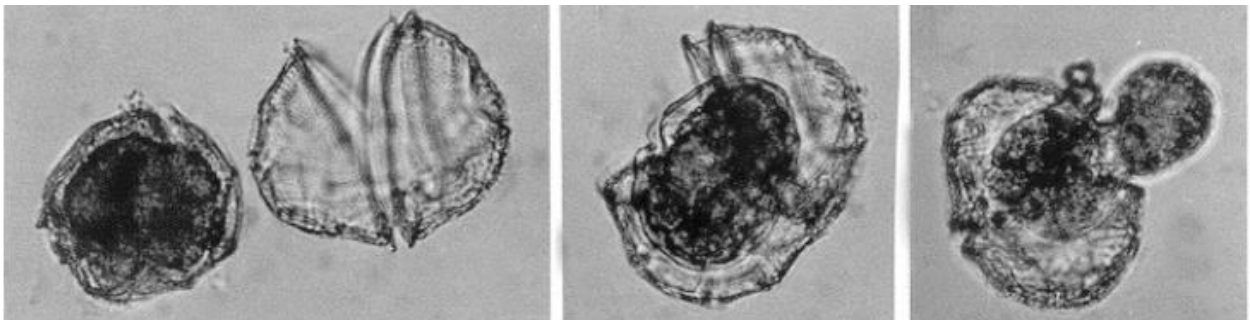

Figure 1.7: *Left* Live cell and theca of *Peridinium gatunense* Nygaard. *Middle* Cell division. *Right* Dead cell with protoplast exiting theca (from Viner-Mozzini et al. 2003).

Pfiester (1975, 1976, 1977) determined that nitrogen deficiency induced sexual reproduction and encystment in *P. gatunense* and *Peridinium willei.* Zohary et al. (2012) recorded the presence of *P.gatunense* in Lake Kinneret (Sea of Galilee, Israel) until the mid-1990s when stable spring blooms became irregular. Laboratory testing revealed that *P.gatunense* densities increased when enriched water flowing from the Jordan River caused the dinoflagellate to bloom (Zohary et al. 2012). Further research determined that hydrological modifications that prevented the Jordan River from flowing into Lake Kinneret regularly affected *P.gatunense* populations. This growth factor from the Hula Valley is likely a microelement and/or an organic compound (Zohary et al. 2012).

Experimental nutrient enrichment on small seepage lakes by Cottingham et al. (1998) determined that phytoplankton communities respond differently to nutrients. In the experiment, Cottingham et al. added phosphates and nitrates to three lakes. *Parvodinium umbonatum* and *Peridinium wisconsinense* were present together with other phytoplankton such as chrysophytes and chlorophytes, and few cyanobacteria were present in 1991 prior to nutrient flux. Following nutrient enrichment, the levels of cyanobacteria began to increase. Over time, the total biomass

of *P. umbonatum* and *P. wisconsinense* decreased as enrichment continued, and by 1994 neither species was present in the eutrophic lakes (Cottingham et al. 1998). Chu et al. (2008) determined the presence of laminated "red tide" layers from Lake Xiaolongwan, NE China coincided with autumn turnover when nutrient levels were high.

1.3.2.3.1 Freshwater dinoflagellate studies: molecular versus morphological evidence

The identity of freshwater cysts and their thecae has often been debated throughout the literature. In the past, morphological evidence was used to identify dinocysts to species (e.g. Norris and McAndrews 1970) and it was difficult to establish a cyst-theca relationship. Recent genetic analysis however has led to advances in dinoflagellate studies. Culturing and DNA sequencing by McCarthy et al. (2011) confirmed the identity of *P.willei* through LSU rDNA analysis. Molecular data from McCarthy et al. (2013) courtesy of Y. Takano suggests that *P. volzii* may be a junior synonym of *P.willei*, as was suggested by Popovsky and Pfeister (1990). Hansen and Flaim (2007) however, propose that cell morphology differs greatly between the two species. *P. willei* and *P. volzii* tend to co-exist in eutrophic conditions and they are differentiated on the basis of size. McCarthy et al. (2013) also suggested that *Peridinium wisconsinense* is closely related to *Chimonodinium* sp. and should probably be reassigned from the genus *Peridinium.*

Cysts (formed after sexual reproduction) will give rise to several individuals with different genotypes (Mertens et al. 2012). Until recently, it was thought that bloom-forming dinocysts are dominated by a few clonal lineages and lacked diversity. DNA studies, however, determined that cysts contain a wide genetic diversity that provides a genetically diverse seed bank that is advantageous during environmental changes (Lorages et al. 2009; Lundholm et al. 2011; Mertens et al. 2012). Kim et al. (2004) studied two genetically distinct but morphologically similar populations of *Peridinium limbatum* (Stokes) Lemmerman found in neighbouring lakes in Wisconsin. Kim et al. (2004) suggest that limited gene flow in lake environments may lead to genetic diversity in the same species leading to morphological similarities, but different genetic structure.

It has been argued that *Parvodinium umbonatum* (Stein) Carty and *Parvodinium inconspicuum* (Lemmerman) Carty are different species due to the genetic variability of specimens based on geographical location (Hansen and Flaim 2007; Carty 2008; Tardio et al.

2009). Lukavsky (2009) grouped *P.umbonatum* and *P. inconspicuum* together based on morphological similarities whereas Carty (2008) suggests that the two species are separate due to the morphological differences in their thecae clearly visible in SEM images (Photo plate 1.1). *P.umbonatum* possesses a deeper and thicker cinglulum and tabulation around the sulcus that is more pronounced (Carty 2008). *P. inconspicuum* is a small (12 - 20 µm in diameter) dinoflagellate that has prominent cingulum and sulcal ridge (Photo plate 1.1). Krueger (2012), however, identified considerable morphological variability in *P. inconspicuum*, particularly a pronounced apical horn and antapical spines. DNA analysis may shed some light on the issue.

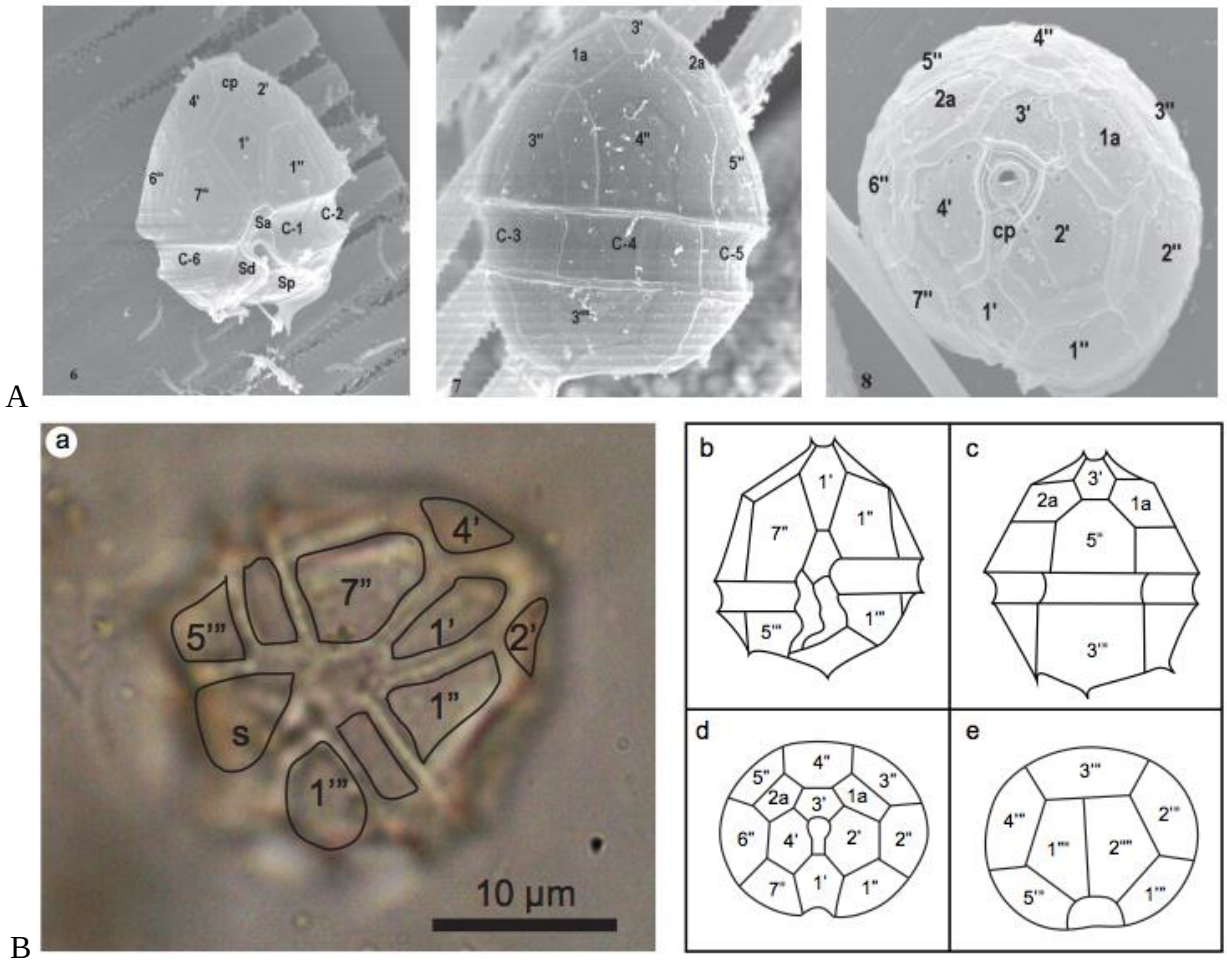

Photo plate 1.1: A) SEM of *Parvodinium umbonatum* (Stein) Carty (from Carty 2008) B) Photomicrograph of *Parvodinium inconspicuum* (Lemmerman) Carty with tabulation (images b - e redrawn from Shen et al. 1990) (from Krueger 2012).

1.3.2.4 Fossil dinoflagellates studies

Resting cysts of freshwater dinoflagellates are found in palynological preparations due to their acid-resistant (dinosporin) composition. Despite their importance in the food chain however, of the approximately 350 freshwater dinoflagellates, only 84 resting cysts have been attributed to species (Mertens et al. 2012 from Moestrup and Calado personal communication). In contrast, marine dinoflagellate cysts have been widely studied and are used in biostratigraphic, paleoecological, and paleoenvironmental reconstructions (e.g. Head 1994; DeSchepper et al. 2009; Mertens et al. 2012).

Unlike cysts, the cellulosic thecae are very rare in the fossil record, but some exceptional environments have led to the preservation of thecae. Thecae have been noted in lagerstatten from Crawford Lake (Krueger 2012; McCarthy and Krueger 2013) and in amber (Masure and Vrielynck 2011). Varved sediments deposited in small, deep and meromictic Crawford Lake contain *Parvodinium inconspicuum* Lemmerman (Carty) thecae in association with abundant small, rounded cysts (16 - 20 µm) in the intervals of Iroquois and Euro-Canadian settlement (Krueger 2012; McCarthy and Krueger 2013) suggesting that this dinoflagellate prefers eutrophic, nutrient-rich conditions.

Cyst morphology may reflect that of the motile cell often displaying paratabulation/parasutures. This cyst type is termed “proximate”. Cysts displaying crests or processes are termed “chorate” or “proximochorate”, depending on the height of the processes or crests (Fensome et al. 1993, 1996; Mertens et al. 2012) (Figure 1.8)

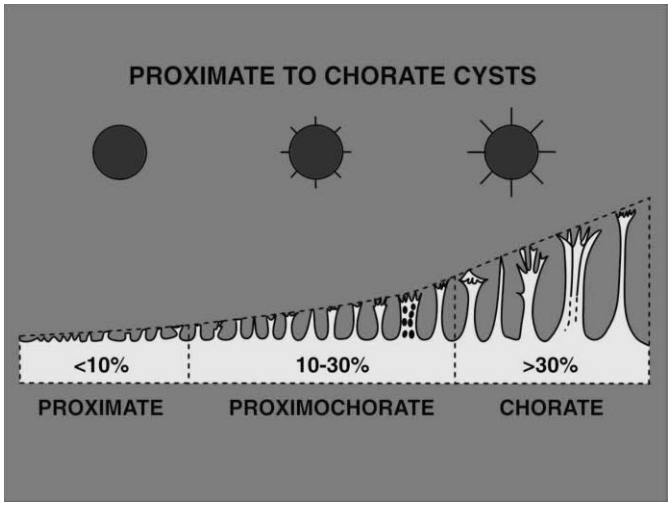

Figure 1.8: “Proximate” to “chorate” describes process/crest length in relation to the shortest diameter of the central body (Fensome et al. 1996).

Peridinium wisconsinense Eddy is large in size (Krueger 2012) and is distinguishable in palynological studies by its cavate, proximate cyst and apical and antapical horns. *Peridinium limbatum* (Stokes) Lemmerman is large (longitudinal length: 45 - 50 μm), with cavate, proximate cysts often displaying paracingulum/paratabulation and one apical horn and two antapical horns (Photo plate 1.2). Cysts attributed to *Peridinium gatunense* Nygaard are large (longitudinal length: 45 μm), rounded cysts and are distinguishable from other freshwater dinoflagellate cysts by clear paratabulation and paracingulum. These cysts were attributed to *P. gatunense* from Pfeister (1977) and Viner-Mozzini et al. (2003) using photoplate comparisons. Thecae of *P. gatunense* are large (36 - 50 x 40 - 47 μm) spherical, with the epi- and hyposome the same size (Hansen and Flaim 2007). Large, ornamented cysts photographed by Pfeister (1977) are similar to cysts attributed to *P. gatunense* in Sluice Pond.

Other common cysts lack obvious morphological characteristics relating them with the motile thecae that produced them. Norris and McAndrews (1970) observed possible cysts of *Peridinium willei* in Minnesota lakes, and tentatively named them Type C and Type D. Culturing studies from Honey Harbour, however, suggested that both Types C and D are *P.willei* (McCarthy et al. 2011). McCarthy et al. (2011) successfully cultured *P.willei* and determined the cyst-theca relationship for this dinoflagellate. Cysts of *Peridinium volzii* spontaneously excysted from varved sediments from Crawford Lake, Ontario (Krueger 2012). Thecae of *P.volzii* are described as ovoid and slightly dorso-ventrally flattened whereas cysts are described as small, unornamented, cavate, and proximate (McCarthy and Krueger 2013). Additional photographs and in-depth cyst descriptions are located in the Appendix, Photoplate 6.1.

A commonly misinterpreted cyst is that of *Chimonodinium* sp. Craveiro et al. It is characterized by an ellipsoidal, thick-walled cyst, often peanut shaped (Craveiro et al. 2011; Mertens et al. 2012).

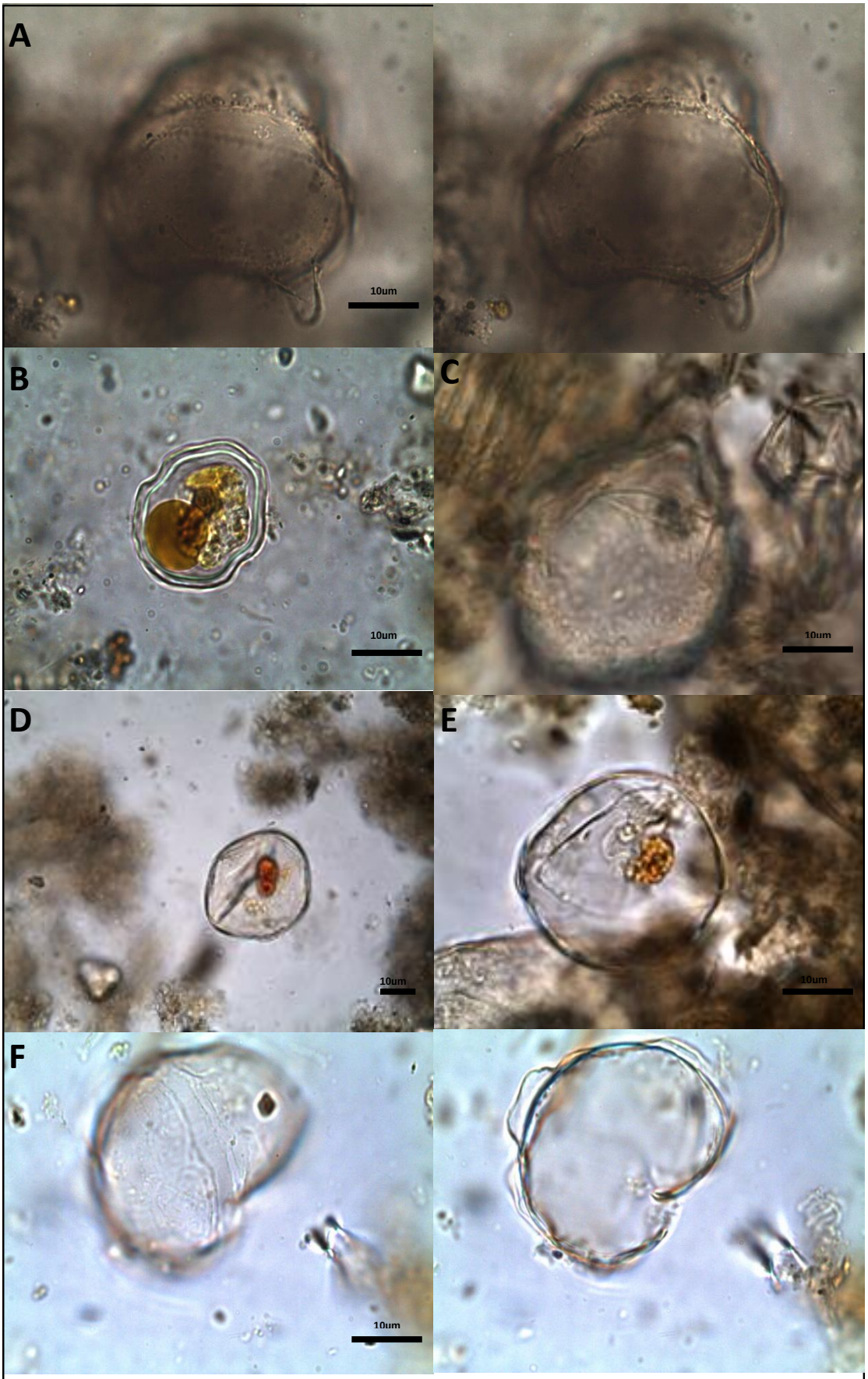

Photo plate 1.2: Common dinoflagellates species in palynological preparations from Sluice Pond. A) Cyst of *Peridinium limbatum* (Stokes) Lemmerman B) Cyst of *Chimonodinium* sp. Craveiro et al. C) Cyst of *Peridinium wisconsinense* Eddy D) *Peridinium volzii* Lemmerman *E) Peridinium willei* Huitfeld-Kaas *F)* Cyst attributed to *Peridinium gatunense* Nygaard. All images were captured at 1000x with the exception of D taken at 400x.

These resting cysts can be germinated, and the cyst-theca relationship can be established (e.g. Wall and Dale, 1968; McCarthy et al. 2011; McCarthy and Krueger 2013). McCarthy et al. (2011) established the cyst-theca relationship for *P.willei* in anthropogenically impacted sediments from Honey Harbour whereas the cyst-theca relationship of *P. volzii* was established by Krueger (2012) from varved sediments of Crawford Lake. *P. volzii* has a rounded theca often containing a red nucleus (35 – 45 μm) whereas *P.willei* cysts are identifiable by their large size (~40 – 50 μm) (Photo plate 1.2).

Small (16-20 μm in diameter), rounded cysts have been attributed to *Parvodinium umbonatum* by both Tardio et al. (2009) and Chu et al. (2008), the latter were shown to be cysts of *Parvodinium inconspicuum* based on the preservation of thecae of this taxon in the same palynological preparations as well as the germination of cysts of this taxon from varves over 100 years old in Crawford Lake (Kueger 2012; McCarthy and Krueger (2013). Tardio et al. (2009) describes cysts of *P. umbonatum* as slightly flattened on the ventral side with the epicyst longer than the hypocyst with paratabulation visible. The paracingulum is visible by a narrowing in the cyst with a displacement slightly towards the hypocyst.

1.3.2.4.1 Dinoflagellates as proxies of cultural eutrophication: evidence from freshwater environments

Studies by Burden et al. (1985) (Figure 1.9) on two cores from Second Lake, Awenda Provincial Park, Ontario found that *Peridinium wisconsinense* responded to intervals of human settlement. However, its peak abundance is associated with pre-human settlement in Second Lake- Core 4. The relative abundance of *P.wisconsinense* decreases as anthropogenic activity in Second Lake-Core 4 continues.

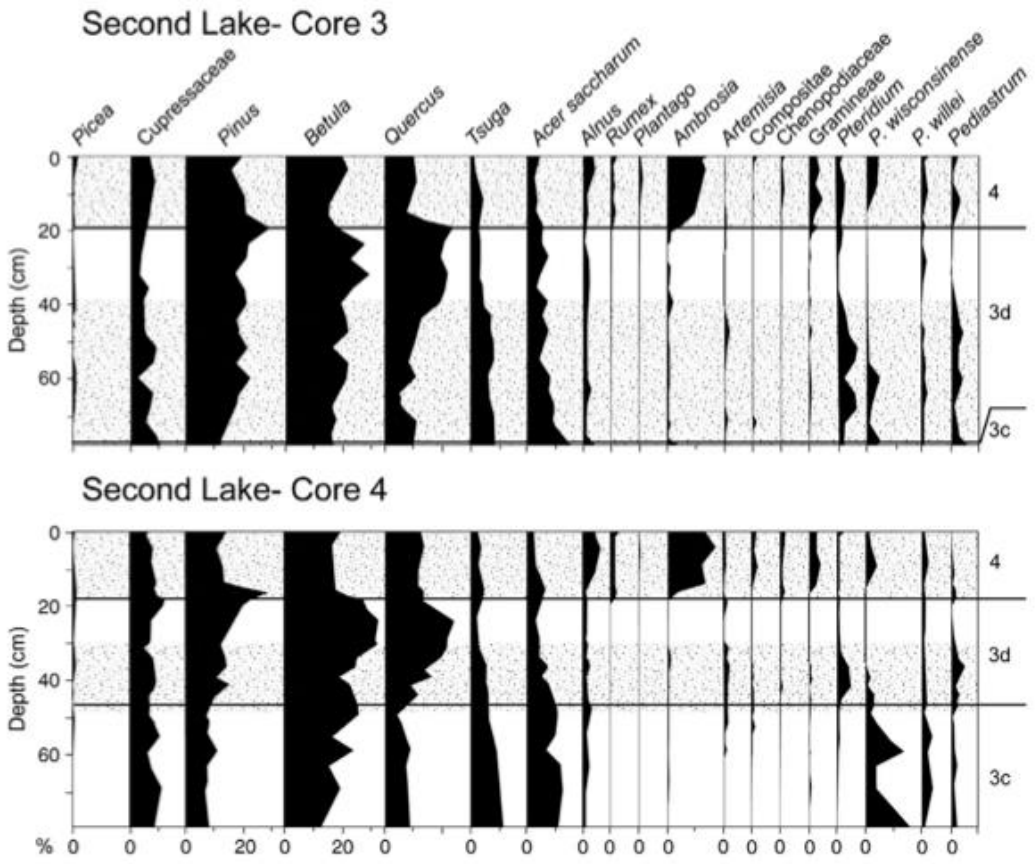

Figure 1.9: Cores analyzed from Second Lake by Burden et al. (1985). Phytoplankton such as dinocysts and *Pediastrum* increased in intervals of human settlement (stippled area). The relative abundance of *Peridinium wisconsinense* Eddy decreased in response to prolonged and peak human impact.

Burden et al. (1985) found a positive relationship between *Peridinium willei* and *Pediastrum* noting that *P.willei* increases during Wendat/ Huronian and Euro-Canadian settlement and responds to an increase in nutrient levels (Figure 1.10). McCarthy et al. (2011) (Figure 1.10) determined that dinoflagellates are useful proxies of cultural eutrophication. In a core taken from Honey Harbour, Georgian Bay, a decrease in absolute abundance of *P. wisconsinense* is observed during Euro-Canadian settlement (AD 1840). *P.willei*, however, dominates (60 - 74% abundance) the *Ambrosia* (ragweed) rich sediments from the upper 20 cm of core taken from Honey Harbour. An increase in terrigenous flux in the upper 20 cm of the core would have increased limiting nutrients such as P and led to a bloom of *P.willei* (McCarthy et al. 2011).

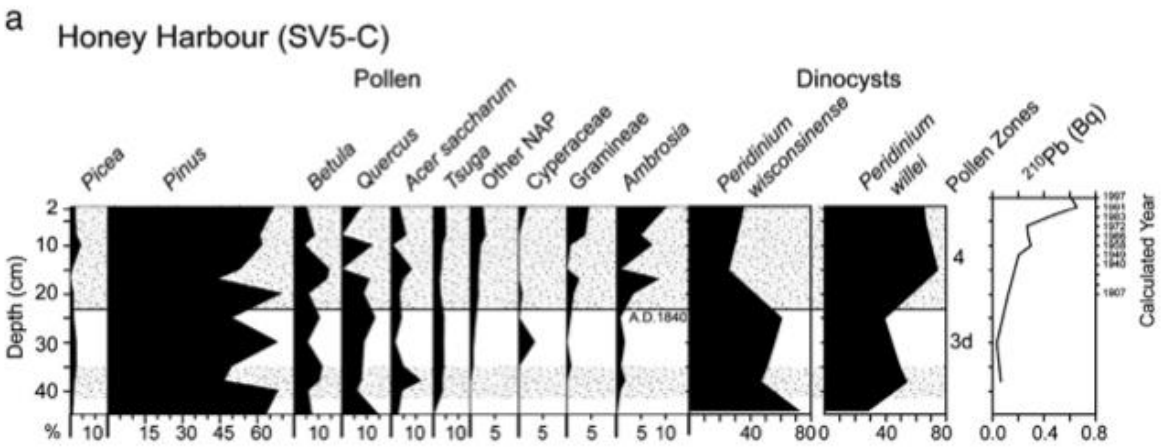

Figure 1.10: Cores from Honey Harbour (Georgian Bay) indicate a decrease in *P.wisconsinense* due to cultural eutrophication (McCarthy et al. 2011).

Zippi et al. (1990; 1991) (Table 1.2) studied 11 lakes in the Haliburton-Muskoka region for paleoindicators of acidification. They found that *P.willei* is cosmopolitan, tolerating a wide range of pH (5.5 - 8.7) and total phosphorus (TP 10.53 ppm). Olrik (1992) also reports that *P. willei* tolerates a broad range of pH, calcium (Ca), chlorine (Cl) and temperature and has the broadest distribution in freshwater lakes. Olrik (1992) states that *P. willei* was present in Swedish lakes with meso/eutrophic conditions, whereas in Norwegian lakes, *P. willei* was most common in oligotrophic lakes. *Peridinium wisconsinense* and *Peridinium limbatum* are attributed to lakes with pH levels ranging from 5.5 - 6.7. For instance, the presence of *P. limbatum* is noted as "very abundant", while *P. wisconsinense,* is "common" in Plastic Lake, an oligotrophic lake in the Canadian Shield (Zippi et al. 1991). *P. limbatum* and *P. wisconsinense* are nonexistent in lakes such as Harp Lake and Little Clear Lake due to sharp increases in total phosphorus (TP) and pH (due to the presence of cottages around the lakes) (Zippi et al.1991).

Table 1.2: Abundances of dinocysts (*P. limbatum- "limb", P. wisconsinense- "wisc", P.bipes- "bipes", P.willei- "willei")* and other palynomorphs taken from the surface sediments of 4 lakes in Haliburton-Muskoka in 1989. AA=very abundant, A=abundant, C=common, P=present. Limnological data (pH, maximum depth *Zmax*, area in hectares A(h), dissolved organic carbon (DOC), and total phosphorus TP) (data from Zippi et al. (1991) and Hall and Smol (1996) compiled in McCarthy et al. 2011; McCarthy et al. 2013)

Lake	pH	*Zmax* (m)	Area(ha)	DOC (mg/l)	TP (mg/Kg)	*limb*	*wisc*	*bipes*	*willei*	*Pedia*	*Zygos*
Plastic	5.7	16.3	32	2.12	4.81	AA	C	C	C		
St. Nora	6.34	39	264	2.97	3.75	C	C	C	P		
Harp	6.36	6.36	542	3.68	6.33				C	C	P
Little Clear	6.82	25	149	2.77	10.53				C	A	

1.3.2.4.2 Exceptional preservation of non-pollen palynomorphs and dinoflagellates in meromictic environments

Crawford Lake is a small (2.4 ha) and deep (24 m) lake whose meromixis has allowed for the exceptional preservation of palynomorphs. Annual layers from varved sediments record Iroquois and Euro-Canadian settlement. Evidence of *Zea* (corn), and grass pollen allowed researchers to determine the presence of an Iroquois village adjacent to the lake (AD 1360). A rise in *Ambrosia* pollen was attributed to Euro-Canadian settlement in the mid 19th century due to clear cutting of the forests (McAndrews and Boyko-Diakonow 1987).

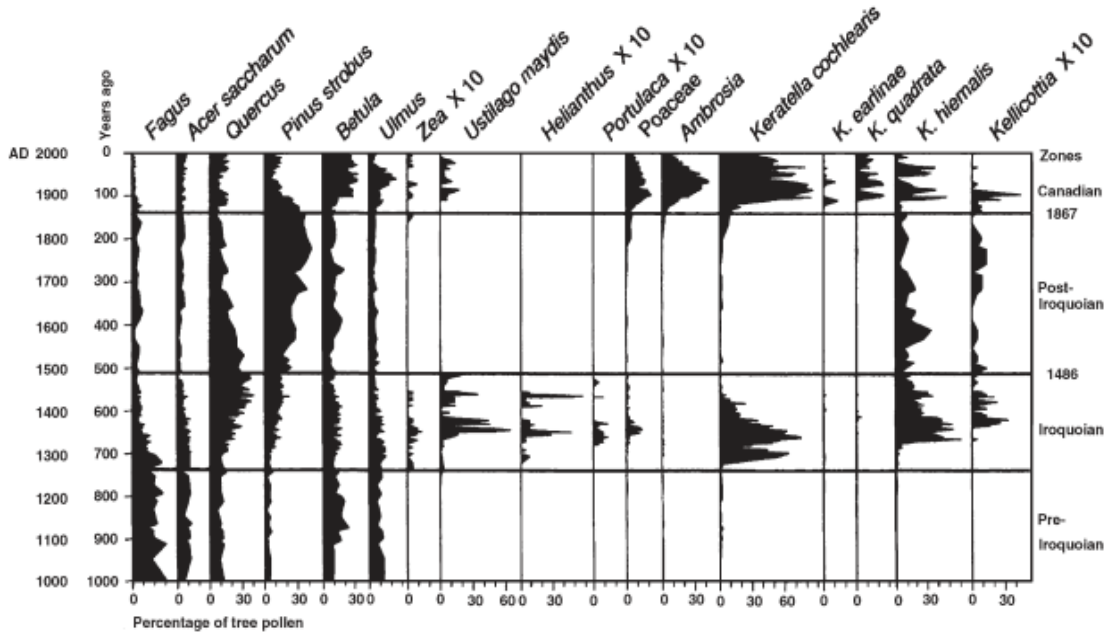

Figure 1.11: Pollen diagrams and rotifer abundance diagrams depicting the initial increase in rotifer lorica during Iroquois settlement. In particular, the rotifer lorica *Keratella cochlearis* decreases in the interval between Iroquois settlement and Euro-Canadian settlement, indicating eutrophic conditions during human settlement (Turton and McAndrews 2006).

Non-pollen palynomorphs (NPP) such as dinoflagellates, desmids, *Pediastrum*, rotifer loricae and various types of protozoa record natural and anthropogenic environmental change in Crawford Lake (Figure 1.11) (Burden et al. 1986; Turton and McAndrews 2006; Danesh 2011; McCarthy et al. 2011; Krueger 2012; Danesh et al. 2013; McCarthy and Krueger 2013; Volik 2014).

Krueger (2012) studied the varved sediments of Crawford Lake and determined that *P. inconspicuum* and *P. volzii* are abundant in sediments that are associated with human settlement. Small, ovoid cysts attributed to *P. inconspicuum* were present in Crawford Lake during Iroquoian settlement from AD 1286 to AD 1486 and Euro-Canadian settlement starting in AD 1841 (Krueger 2012) (Figure 1.12).

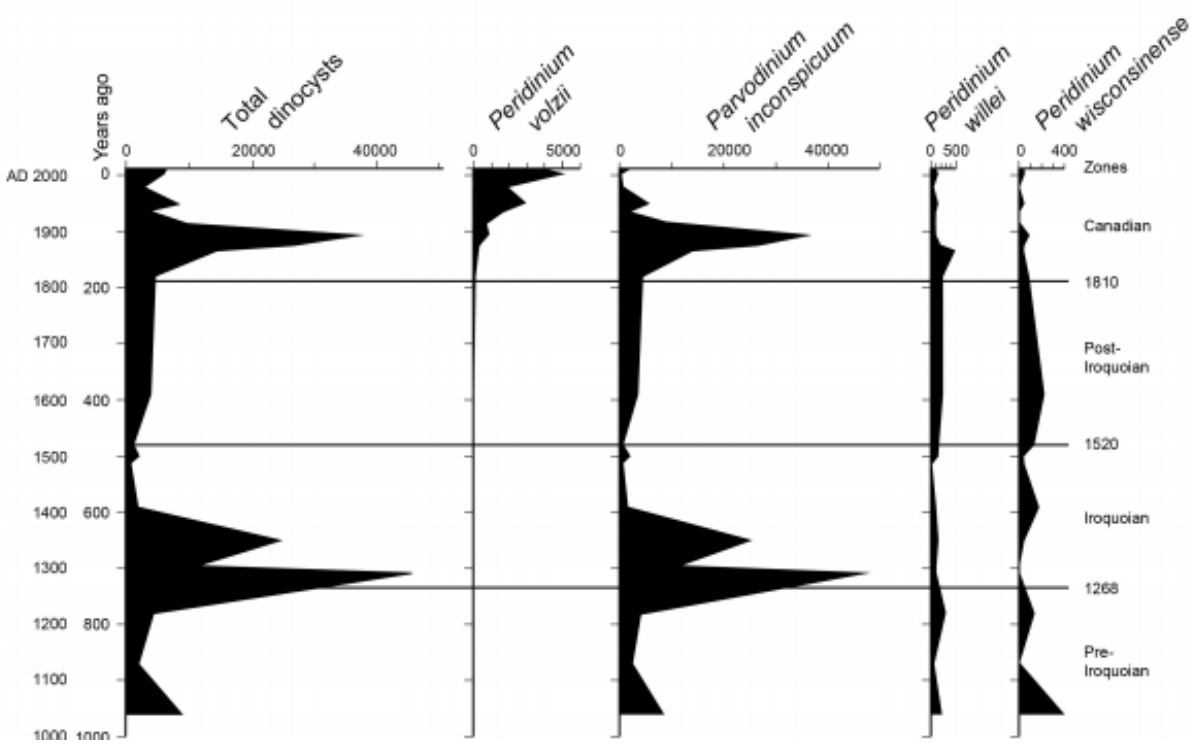

Figure 1.12: Exceptionally preserved, varved, sediments from Crawford Lake, Ontario depict cysts attributed to *Peridinium volzii* Lemmerman, *Peridinium willei* Huitfeld-Kaas, *Peridinium wisconsinense* Eddy, and cysts and thecae of *Peridinium inconspicuum* Lemmerman. Peak cyst and theca abundances of *P. inconspicuum* are associated with Iroquoian and Euro-Canadian settlement whereas peak cyst abundance of *P.wisconsinense* is attributed to the interval between Iroquoian and Euro-Canadian settlement. The appearance of *P. volzii* is attributed to fish stocking as a result of Euro-Canadian settlement. Euro-Canadian and Iroquoian zones are determined using pollen of cultivars (from Krueger 2012; McCarthy and Krueger 2013).

P.inconspicuum is found in freshwater lakes, ponds and marshes and is most abundant in low pH (Krueger 2012). Krueger postulates that the presence of *P.volzii* in the upper 32cm of core is attributed to the introduction of sport fish to Crawford Lake after Euro-Canadian settlement. Consequently, Carty (2003) and Carty and Fazio (1997) both attributed the presence of *P.volzii* to nutrient-enriched water in North American and Europe.

1.4 An introduction to thecamboebians and ciliates

Thecamoebians (testate amoebae) are unicellular agglutinated protists found in freshwater environments such as lakes, rivers, ponds and sufficiently moist habitats such as wetlands and bogs (Ogden and Hedley 1980). These epifaunal/shallow-infaunal benthic protozoans are abundant in the Quaternary to modern lacustrine and wetland sediments, however they range

back to Neoproterozoic Chuar Group, Grand Canyon (Porter and Knoll 2000; Neville et al. 2011).They are also common in brackish environments such as salt marshes and estuaries (Medioli and Scott 1988). They are a diverse group of protists belonging to two different Classes and several Orders within the Phylum Sarcodaria. Thecamoebians belong to Class Rhizopodea von Siebold, Order Arcellinidia Kent, Superfamily Arcellacea Ehrenberg. Most thecamoebians can be sub-divided on the basis of their family; the two most common families in aquatic environments: Centropyxidae Jung and Difflugiidae Stein.

1.4.1 Systematic taxonomy of common thecamoebians and ciliates

Phylum	Sarcodaria Milne-Edwards 1850
Superclass	Rhizopoda Dujardin 1835
Class	Lobosa Carpenter 1861
Subclass	Testacealobosa de Saedeleer 1934
Order	Thecolobosa Haeckel 1878
Superfamily	Arcellacea Ehrenberg 1830
Family	Difflugidae Stein 1859
Genus	*Cucurbitella* Carter 1856
Species	*C. tricuspis* (Carter) Medioli et al. 1987
Genus	*Difflugia* Leclerc *in* Lamark 1816
Species	*D. amphora* Wallich 1864
	D. bacillariarum Perty 1849
	D. bidens Penard 1902
	D. corona Wallich 1864
	D. fragosa Hempel 1898
	D. globulus Ehrenberg 1848
	D. oblonga Ehrenberg 1832
	D. protaeiformis Lamarck 1816

	D. urceolata Carter 1864
Genus	*Lagenodifflugia* Leidy 1874
Species	*L. vas* Leidy 1874
Genus	*Lesquereusia* Schlumberger 1845
Species	*L. spiralis* (Ehrenberg 1840)
Genus	*Pontigulasia* Rhumbler 1895
Species	*P. compressa* (Carter 1864)
Family	Centropyxidae Deflandre 1953
Genus	*Centropyxis* Stein 1859
Species	*C. aculeata* (Ehrenberg 1832)
	C. constricta (Ehrenberg 1843)
Family	Arcellidae Ehrenberg 1830
Genus	*Arcella* Ehrenberg 1830
Species	*A. vulgaris* Ehrenberg 1830
Family	Hyalospheniidae Schulze 1877
Genus	*Heleopera* Leidy 1879
Species	*H. sphagni* Leidy 1879

Phylum	Chromista Cavalier-Smith 1981
Subphylum	Intramacronucleata Lynn 1966
Class	Spirotrichea Small and Lynn 1985
Order	Tintinnida Kofoid and Campbell 1929
Family	Codonellidae Kent 1882
Genus	*Codonella* Haeckel 1873
Species	*C. cratera* Leidy 1877

1.4.2 Protozoans and their usefulness in paleolimnological studies

Most thecamoebians build their tests by agglutining foreign particles with an autogenous cement, thereby creating a xenogenous test (Kumar and Patterson 2000). The composition of these tests varies based on the composition of the substrate itself, and may consist of diatoms or sand grains (Medioli et al. 1990; Kumar and Patterson 2000). Most thecamoebians produce a fossilizable test containing pseudochitinous material and agglutinated particles (Medioli et al. 1983; Neville et al. 2011). The availability and composition of foreign particles affects the appearance of the thecamoebian itself. McCarthy (1984) notes that specimens of *Centropyxis aculeata* Ehrenberg incorporated grains of carborundum in their tests while Patterson et al. (1996) notes the incorporation of shiny metallic particles in xenosomes from heavy metal mining lakes in northern Ontario. These morphological differences and their rapid asexual morphing (usually in the order of only a few days) in response to environmental stresses make thecamoebians excellent indicators of an ecosystem's health (Ogden and Hedley 1980; Reinhardt et al. 1998; Kumar and Patterson 2000; Neville et al. 2011).

Difflugiid thecamoebian tests are either flattened or sphaerical with an aperture located at the tapered end. Centropyxid thecamoebians are doughnut-shaped with an invaginated aperture located on the ventral side (Medioli and Scott 1983; Neville 2010). Thecamoebians feed on bacteria, algae, and fungi; however some species prey on other protozoa (Ogden and Hedley 1980). Difflugiid thecamoebians primarily feed on algae but centopyxid thecamoebians are bacteriophage (McCarthy 1984) allowing them to thrive in harsh environments.

Thecamoebians are excellent indicators of stressed conditions, cultural eutrophication and overall changes in the environment (Kumar et al. 1999). Seasonal studies on the surface sediments of reclamation ponds of Suncor Energy Inc. determined that chemical composition, seasonal temperatures, pH, and dissolved oxygen (DO) influenced thecamoebian assemblages (Neville et al, 2010; Neville et al., 2011). Thecamoebians are useful in paleoenvironmental studies since they morph asexually due to environmental stresses (Reinhardt et al. 1998). These strains (or morphotypes) can be used in paleo-environmental analysis (Dalby et al. 2000). Thecamoebians have been used extensively in paleoenvironmental reconstructions alongside

pollen and other proxies (McCarthy et al. 1995; McCarthy et al. 2012; Danesh et al. 2013) (Figure 1.13).

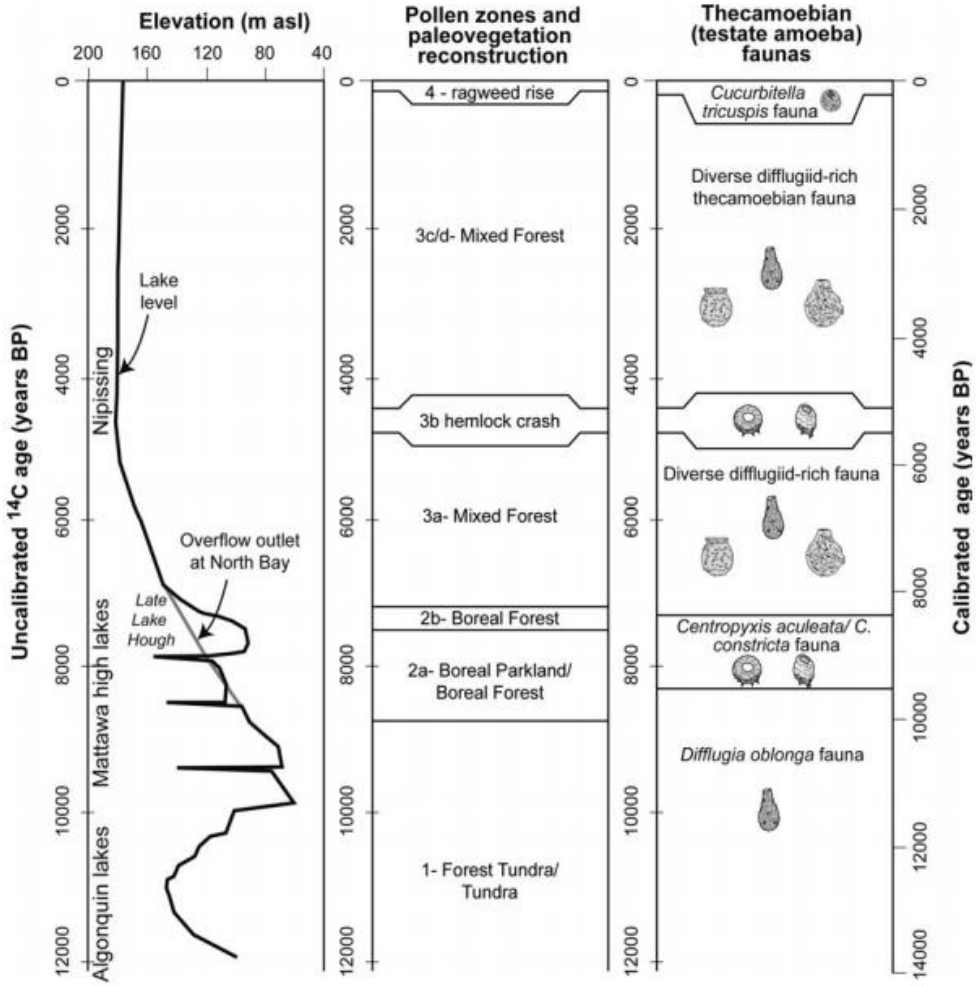

Figure 1.13: A summary diagram from McCarthy et al. (2012) depicting lake level, pollen zonations and thecamoebian abundances in the Georgian Bay basin. The establishment of centropyxids in the closed basin following deglaciation during the boreal parkland records harsh conditions. Following the establishment of warm and wet conditions (mixed forest), difflugiids become established. This is perturbed by the hemlock minimum where dry conditions created a harsh environment. The human impact zone is characterized by a rise in ragweed pollen and the dominance of *Cucurbitella tricuspis* (Carter) Medioli et al.

Patterson et al. (1996) determined that assemblages containing *Centropyxis aculeata* Ehrenberg, *Centropyxis constricta* Ehrenberg and *Arcella vulgaris* Ehrenberg were present in northeastern Ontario lakes with high levels of As and Hg loading whereas undisturbed lakes contained higher populations of *Cucurbitella tricuspis* (Carter) Medioli et al. The presence of

A.vulgaris is known to exist in high organic carbon (OC) and low DO environments (Reinhardt et al. 1998). Torigai et al. (2000) on Lake Winnipeg determined the density of *C. tricuspis* is associated with heavy metal loading into the Assiniboine-Red River. *C. tricuspis* escapes heavy metal pollution by attaching itself to floating algae near the water's surface (Torigai et al. 2000). Roe et al. (2010) determined the affinity for certain thecamoebians to DO levels by examining 71 surface samples from 21 Greater Toronto Area (GTA) lakes. Roe et al. (2010) determined that assemblages of centropyxids, *C.tricuspis, D.oblonga* and *D. urceolata* were present in low oxygen lakes, while high populations of *C.tricuspis* indicated eutrophic algal assemblages (Figure 1.14).

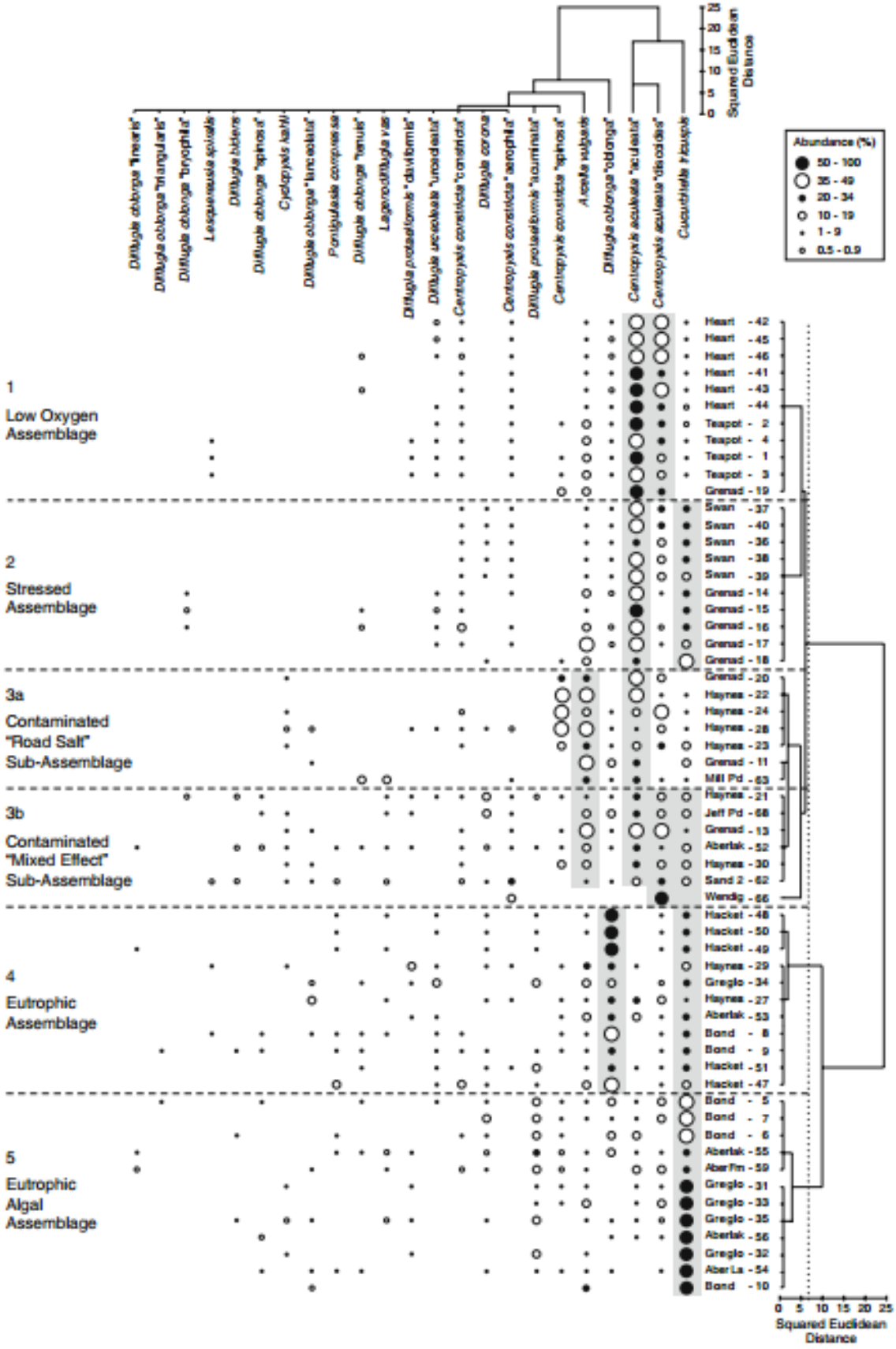
Squared Euclidean Distance
Abundance (%)
50 - 100
35 - 49
20 - 34
10 - 19
1 - 9
0.5 - 0.9
Diffugia oblonga "linearis"
Diffugia oblonga "triangularis"
Diffugia oblonga "bryophila"
Lesquereusia spiralis
Diffugia bidens
Diffugia oblonga "aphnos"
Cyclopyxis kahli
Diffugia oblonga "lanceolata"
Penisutella compressa
Diffugia oblonga "tenuis"
Lagenodiffugia vas
Diffugia protaeiformis "daviformis"
Diffugia urceolata "unoidalis"
Centropyxis constricta "constricta"
Diffugia corona
Centropyxis constricta "aerophila"
Diffugia protaeiformis "acuminata"
Centropyxis constricta "aphnos"
Arcella vulgaris
Diffugia oblonga "oblonga"
Centropyxis aculeata "aculeata"
Centropyxis aculeata "discoides"
Cucurbitella tricuspis
1
Low Oxygen Assemblage
2
Stressed Assemblage
3a
Contaminated "Road Salt" Sub-Assemblage
3b
Contaminated "Mixed Effect" Sub-Assemblage
4
Eutrophic Assemblage
5
Eutrophic Algal Assemblage
Heart - 42
Heart - 45
Heart - 46
Heart - 41
Heart - 43
Heart - 44
Teapot - 2
Teapot - 4
Teapot - 1
Teapot - 3
Grenad - 19
Swan - 37
Swan - 40
Swan - 36
Swan - 38
Swan - 39
Grenad - 14
Grenad - 15
Grenad - 16
Grenad - 17
Grenad - 18
Grenad - 20
Haynes - 22
Haynes - 24
Haynes - 26
Haynes - 23
Grenad - 11
Mill Pd - 63
Haynes - 21
Jeff Pd - 68
Grenad - 13
Aberlak - 52
Haynes - 30
Sand 2 - 62
Wendig - 66
Hacket - 48
Hacket - 50
Hacket - 49
Haynes - 29
Greglo - 34
Haynes - 27
Aberlak - 53
Bland - 8
Bland - 9
Hacket - 51
Hacket - 47
Bland - 5
Bland - 7
Bland - 6
Aberlak - 55
AberFm - 59
Greglo - 31
Greglo - 33
Greglo - 35
Aberlak - 56
Greglo - 32
AberLa - 54
Bland - 10
0 5 10 15 20 25
Squared Euclidean Distance

Figure 1.14: Roe et al. (2010) determined the presence of *Centropyxis aculeata* Ehrenberg, *Centropyxis constricta* Ehrenberg, *Cucurbitella tricuspis* (Carter) Medioli et al., *Difflugia oblonga* Ehrenberg reflect low oxygen environments in Greater Toronto Area (GTA) lakes (from Roe et al. 2010).

McCarthy et al. (1995) determined that post-deglaciation environments were ideal for *C. aculeata* as it could survive hostile conditions. *C.aculeata* populations decreased as *P.compressa* increased during the warm Hypsithermal, in organic-rich sediments. During the late Holocene (from 4,000 to 1,000 years BP), as climatic cooling occurred, *C. aculeata* increased again (McCarthy et al. 1995) (Figure 1.15).

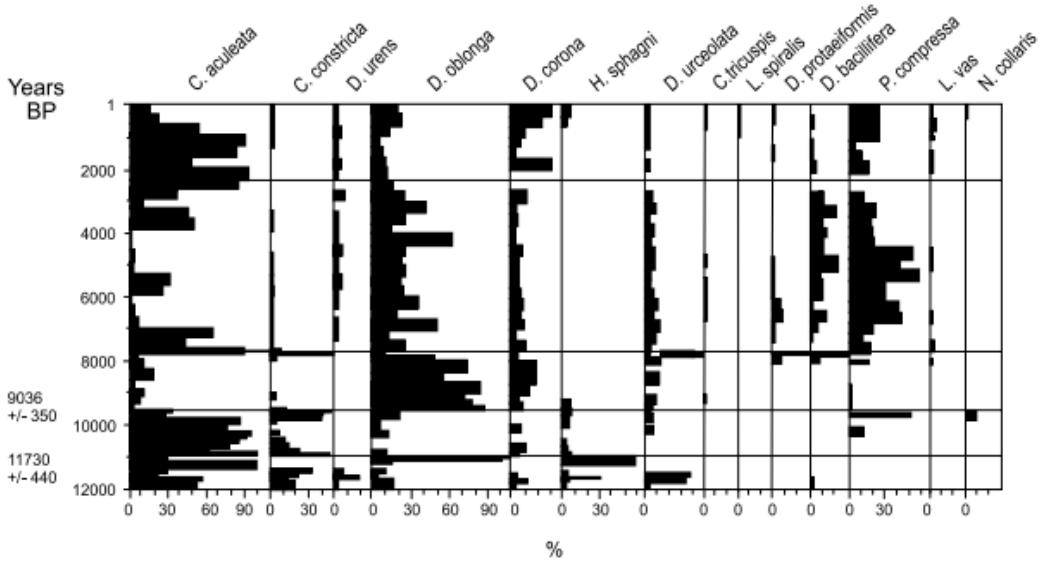

Figure 1.15: Thecamoebian data from Penhorn Lake, Nova Scotia (from McCarthy et al. 1995) modified by Patterson and Kumar (2002). Following the retreat of the Laurentide Ice Sheet, populations of *Centropyxis aculeata* and *Centropyxis constricta* dominated harsh environments.

Ciliates are a basic component of microplankton and microbenthic communities. They play a critical role at the base of the food chain in many aquatic environments. Ciliate assemblages often contain species that are sensitive to pollutants (Madoni 1999). Certain species exhibit tolerance towards pollutants and can be used as indicators of ecosystem health. Ciliates are similar to thecamoebians in that they are both protozoa. *Codonella cratera* Leidy are vase-shaped tintinnids that are often seen in thecamoebian analysis. Studies on the Rio Grande reservoir in Brazil determined that *C. cratera* was observed in areas of increased eutrophication, in anoxic, muddy bottom waters (Barbieri and Orlandi 1989). Danesh et al. (2013) noted the

presence of *C. cratera* in association with a rise in heavy metals and phosphorus due to anthropogenic impact. Limon et al. (1983) notes the presence of *C.cratera* in a heavily populated area surrounding Lake Cajititlan, Mexico. Increased trophic status of Kabetogama Lake, Minnesota (Kling 2005) indicates that *C. cratera* is often present in turbid, riverine-type environments and peaks in sediments from the 1920s, likely associated with a rise in industry. Further studies on microfossil communities indicate that heavy metals can affect microplankton and zooplankton communities (Moore et al. 1979). Bioaccumulation of Pb and Cd lowered protozoan populations in marine studies (Fernandez-Leborans and Herrero 2000). El-Basat and Taylor (2007) studied Abo Zabaabal, a new mining lake near Cairo, Egypt that receives domestic sewage after tilapia stocking failed and noted the presence of high populations of *C. cratera* in this highly eutrophic lake. Further studies by Taticchi (1992) on Lake Trasimeno and other water bodies of the Umbria region in Italy determined that *C.cratera* was dominant from January 1990 to June 1991 when TP ranged from 0 - 170 µg/L and total N ranged from ~1 - 2.7 mg/L. This area's greatest environmental impact is the hog farming industry, which inevitably has an effect on the lakes in the area.

1.5 A brief history of Lynn, MA

The city of Lynn was settled in AD 1629 by Puritans from England. The settlement, like many in New England, relied on Boston for commerce as Lynn itself did not contain a natural harbour or deep water to moor large trading vessels. Lynn was predominantly swampy, rocky land with sand beaches along the coast. Due to Lynn's lack of harbour, ships carrying goods from the West Indies, Africa and Europe did not frequent the settlement. Therefore, the need for professional services such as banks, booksellers, newspapers, and schools for children over the age of 12 was not established until the 19th century (Faler 1981).

From AD 1630-1750, Lynn remained a struggling agricultural village, with its townspeople combing beaches for organic matter or stripping the rich humus from the topsoil of the marsh to enrich the soil. Lynn was surrounded by extensive marsh on the western portion of the settlement, stretching across to the Saugus River and was periodically inundated by ocean tides. The flat plain that receded to the sea was untillable earth. The little food produced by agriculture could only sustain a small village. From AD 1640-1740, the population was stagnant, and in AD 1765, only 1798 people lived in Lynn (Faler 1981).

The rise of the shoe industry transformed Lynn. Vessels from overseas returned to Boston or Salem with raw materials such as leather, which Lynn residents fashioned into shoes. As the enterprise grew, Lynn obtained cowhides from the West Indies, Philadelphia, Baltimore, and later California and Latin America (Faler 1981). Little is known about the slow-growing shoe business until AD 1750. In AD 1768, 80,000 pairs of shoes were manufactured in Lynn.

Lynn's manufacturing sector grew slowly as England discouraged colonial production due to fears it would lead to autonomy. English shoes were also more popular as their wares were cheaper and better made. In AD 1750, John Adam Dagyr, a Welsh shoe-maker, transformed Lynn's industry by dissecting the English goods and improving the quality of Lynn-made shoes. The turning point in Lynn's fortunes came during the American Revolution when British goods were boycotted. This caused shoe production to increase from 80,000 pairs in AD 1768 to 400,000 in AD 1783. By AD 1763, there were 50 shoemakers in Lynn, with whole families employed to make shoes. As the American Revolution ended, a tariff was placed on English goods, allowing Lynn to establish itself as an important manufacturing town (Faler 1981). Lynn was a popular manufacturing town well into the 20th century. In AD 1930, the population of Lynn peaked to 102,320 residents- an increase of over 90,000 residents in 90 years (United States Census Survey 2010).

1.5.1 Leather Production in New England

The city of Woburn, Massachusetts is approximately 26 km east from the city of Lynn. Like Lynn, it flourished in the late AD 1600s from the tanning industry. According to Tarr (1987), by the mid- 1800s to mid-1900s, over 100 leather tanning factories were in operation on the Aberjona River. In 1850, Woburn Chemical Works was established and began the production of chemicals for New England's tanning industry. Between AD 1853 and AD 1933, the Merrimac Chemical Company (successor of Woburn Chemical Works) also produced Ar and Pb based pesticides, sulfuric acid, and acetic acid used in the leather tanning industry. In the late 19th century, pyrite ore was used as a source of sulphur (S) and was burned on-site. The pyrite ore contained several heavy metals such as Pb, Hg and Cr. Arsenic sourced from pyrite lead to the release of 200 to 900 metric tons of pollution into the Aberjona River (Tarr 1987). These chemicals (used in tanning practices) were likely transported and used in Lynn due to the city's

proximity to Woburn. Woburn's well-documented history is a proxy for Lynn's leather tanning industry.

Leather tanning is a general term used to describe the process of producing leather from the acquisition of livestock to the end product. Animal skins were first cured in brine for several hours to days to remove fat and excess flesh. Following the brining, the skin was limed to remove hair. Following the liming process, the skin was de-limed in an acid vat and exposed to enzymes to produce a supple texture (http://serc.carleton.edu/woburn/issues/tanning.html). Following this process, the leather was "tanned" in vegetable tannins (naturally found in chestnut, hemlock and oak trees). By the early 1900s however, the process of pickling hides with chromium salts became more favourable. Following this tanning process, many hides were bleached, or rolled mechanically to produce softer leather.

1.5.2 Geologic setting

The surrounding geology consists of Precambrian-cored Avalon Terrane (accreted during the Acadian Orogeny) and is underlain by the northwest-southeast trending Walden Pond fault (Hon and Hanson 2004; Zen et al. 1983). Within the watershed of Sluice Pond, the geology consists of Proterozoic "Salem" gabbro-diorite, and the Devonian-aged Peabody Granite (to the north) and Proterozoic-Devonian-aged Lynn Volcanics complex and the Proterozoic Dedham Granite (Hubeny et al. in press) (Figure 1.16).

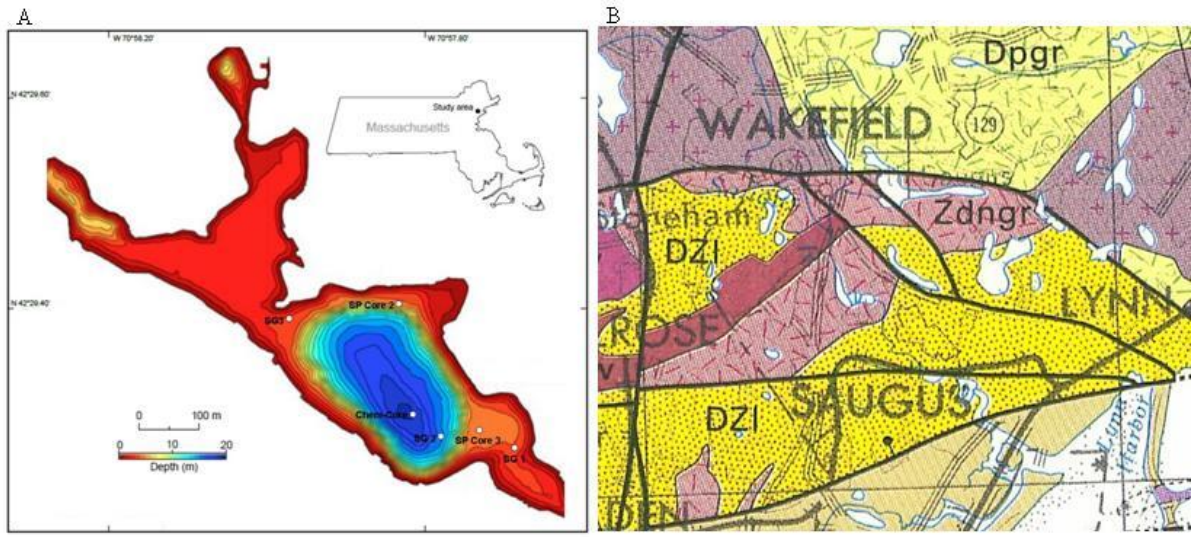

Figure 1.16: A) Bathymetric map of Sluice Pond with 2 m contours showing the meromictic deep basin (from Hubeny et al. in press) B) Sluice Pond lies between a fault line and is

comprised of DZI- Lynn volcanic complex (Lower Devonian- Silurian) rhyolite (yellow stippled), agglomerate and tuff to the south, Zdngr- grey granite to grandiorite to the northwest and Zdigb (purple crosses)- diorite and gabbro (Proterozoic Z) complex of diorite and gabbro, subordinate metavolcanic rocks and intrusive granite and granodiorite to the northeast and Dpgr- Peadbody granite- to the north (Zen et al. 1983) (from Hubeny et al. in press).

The inflow of the lake is controlled by several small streams to the north, northwest and street run-off and the outflow is controlled by a dammed stream in the southeast tip of the lake (Hubeny, et al., submitted). According to Randall et al. (2008), the lake is a part of a group of glacially-formed lakes that are fed by groundwater flowing along bedrock fractures into the Atlantic Ocean.

1.5.3 A multi-proxy study of Sluice Pond by Hubeny et al. (in press)

Seven seismic reflectors were identified in the deep basin (19.7 m) core SP09 KC2 with the bottom pink reflector representing the hummocky surface of the Boston Blue Clay glacio-marine unit (Figure 1.17) (Kaye, 1978). The lower portion of the core (467 cm) represents 11.41 ± 0.21 ka. The seismic sediment packages overlying the pink reflector vary in thickness from ~1.5 - 6m and represent lacustrine sedimentation in the late Pleistocene. The lowest two reflectors (overlying the pink reflector), red and light blue, are restricted to core SP09 KC2 in the lake's deep basin. The red reflector pinches out at ~23.5 m below modern lake level and the light blue pinches out at 23 m below modern lake level. The seismic reflector above the light blue, dark blue, has a maximum elevation of ~18.5 m below modern lake level. The maroon reflector (286 - cm, 7.42 ± 0.09 ka) exhibits a draping profile across the lake and pinches out at ~15.5 m bmll. Above the maroon reflector, the green reflector also exhibits a draping pattern throughout the pond and pinches out at ~14 m below modern lake level. The sediment water interface is represented by the last seismic package- yellow. Both green and yellow reflectors are of variable thickness throughout the pond.

Marginal core SP07 PC4 contains gray, sandy clay, interpreted as the Boston Blue Clay with the top of this lithofacies corresponding to the pink reflector (16.5 ± 0.7 ka). The next visible seismic reflector in the marginal core is the maroon reflector (7.81 ± 0.16 ka), indicating a hiatus between Holocene and late Pleistocene sediments. Pollen confirms a hiatus in the middle Holocene associated with the hemlock minimum. The green reflector overlies the maroon and is

organic-rich with abundant macrophyte remains, followed by the sediment water interface reflector- yellow (Hubeny et al. in press).

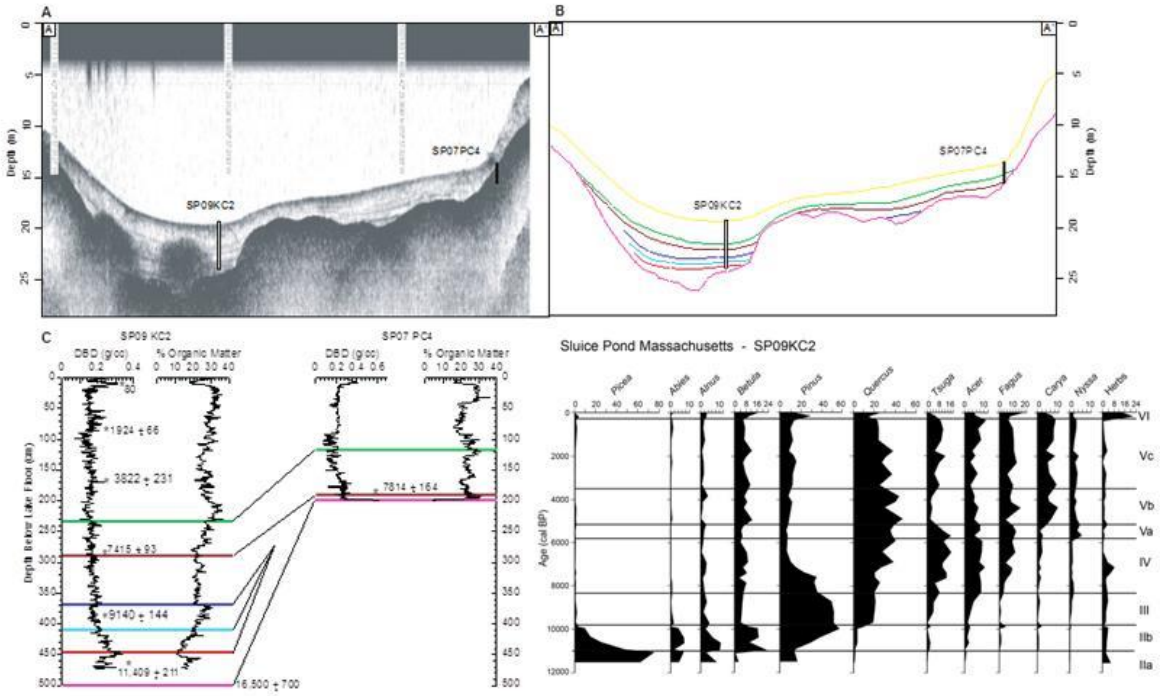

Figure 1.17: Seismic reflectors, pollen data (Analyst: Dr. F. McCarthy), DBD, %OM and ^{14}C radio carbon ages used to chronologically constrain the cores (Hubeny et al. in press).

Pollen stratigraphy and five AMS ^{14}C dates on core SP09 KC2 and one for core SP07 PC4 using terrestrial macrofossils with an acid-base acid (ABA) procedure (Olsson 1986) were calibrated using CALIB 6.0 program and Intcal09 calibration data set. Other physical and organic proxies were also used- magnetic susceptibility (ƙ), dry bulk density (DBD), organic carbon (OC%; black curve from LOI analyses, gray curve from EA analyses), stable carbon isotopic composition ($\delta^{13}C$), organic carbon: nitrogen ratio (OC/N), stable nitrogen isotopic composition ($\delta^{15}N$), stable sulfur isotopic composition ($\delta^{34}S$), organic carbon: sulfur ratio (OC/S), total nitrogen (N%), and total sulfur (S%) (Hubeny et al. in press). The radiometric chronology was compared to the pollen stratigraphy using well dated regional pollen data (Appendix, Figure 5.3) (Newby et al. 2000; Shuman et al. 2002; Webb et al. 2004).

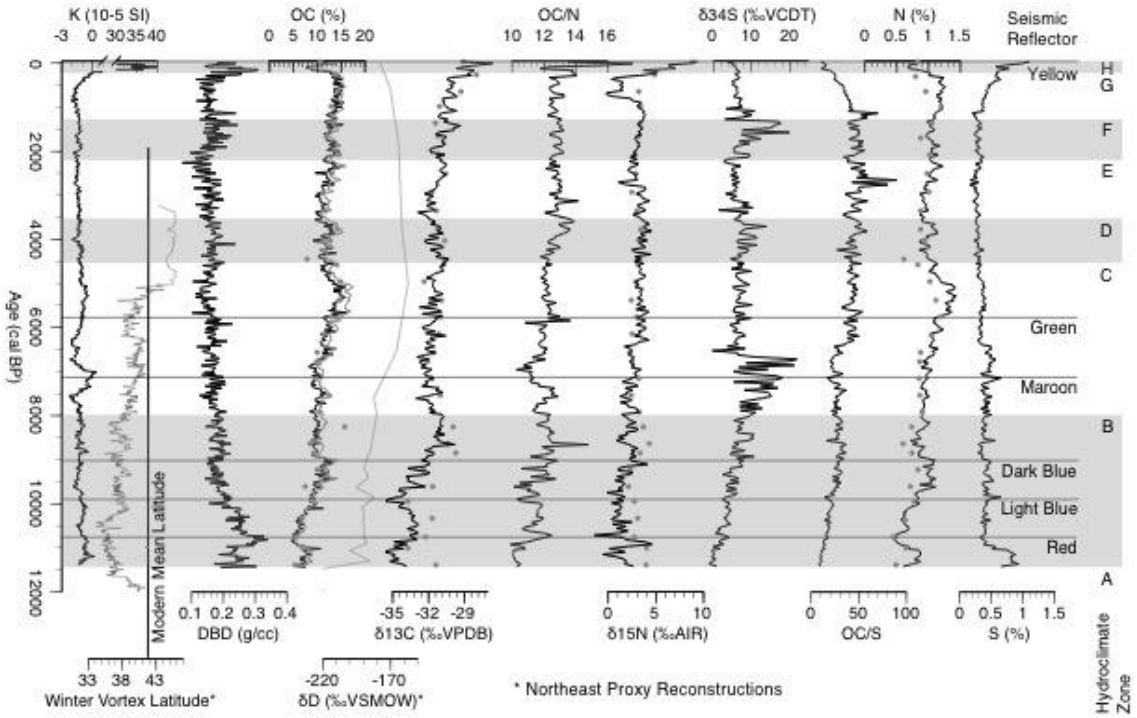

Figure 1.18: Physical and organic proxies for core SP09. From left to right: magnetic susceptibility (ƙ), dry bulk density (DBD), organic carbon (OC%; black curve from LOI analyses, gray curve from EA analyses), stable carbon isotopic composition ($\delta^{13}C$), organic carbon: nitrogen ratio (OC/N), stable nitrogen isotopic composition ($\delta^{15}N$), stable sulfur isotopic composition ($\delta^{34}S$), organic carbon: sulfur ratio (OC/S), total nitrogen (N%), and total sulfur (S%) (Hubeny et al. in press).

Ƙ values are low throughout most of the core indicating high inputs or organic material, low in silisiclasic sediments (Figure 1.18). In the anthropogenic zone, ƙ rises due increases in industrial activity and the combustion of fossil fuels. DBD values are relatively low due to large amounts of water and organic matter in the sediment. DBD increases in the anthropogenic zone as watershed input into the lake increases. OC% was completed at 1 cm increments using LOI and spectral analysis. OC% suggests past productivity within the water column. A decrease during the hemlock minimum is slightly visible in the figure, corresponding to Shuman et al. (2001). A decrease at the top of the core due to anthropogenic impact suggests increased terrigenous inputs from land-use changes. C/N analysis suggests that lacustrine algae have dominated the basin of Sluice Pond (C/N values for lacustrine algae =4 - 10, vascular plants= >20) (Meyers and Lallier-Vergès, 1999). $\delta^{13}C$ values average -31.71‰, suggesting dominance of C4 plants during the Holocene whereas the $\delta^{15}N$ values average 1.63‰ with the topmost sediments reaching 9.37‰

due to an input of animal waste and fertilizers. OC/S ratios average 34.10 indicating lacustrine sediments. An increase is observed at 469 cm (11.36 ka) where glaciomarine sediments may have provided re-worked sulfates and near the surface where the burning of fossil fuels increased atmospheric precipitation of sulphates (Hubeny et al. in press).

1.5.4 Lake geometry and affinity for meromixis in Sluice Pond

Lake geometry plays a vital role in understanding past environments and future environments in lake systems. The frequency of turnover in man-made and natural lakes is largely controlled by solar and wind energy. At the start of spring, the water column has a uniform temperature (and thus density), allowing mixing to occur in "holomictic" lakes (Castendyk 2009). Wind energy promotes further mixing (Figure 1.19). As spring progresses, increased solar radiation increases the temperature and decreases the density of the surface layer relative to the water below it and "summer stratification" results until fall, when turnover commences as a result of temperature change of the surface waters, and subsequent increase in density. This density change causes the surface waters to sink, breaking down stratification.

Lakes which do not experience turnover are permanently stratified and are termed "meromictic", consisting of monimolimnion, chemocline, and the mixolimnion (Castendyk 2009). The monimolimnion is the deepest part of the lake and is generally anoxic and/or saline. The mixolimnion is the upper layer of water near the surface which mixes similar to a holomicitc lake while the area between the monimolimnion and mixolimnion is referred to as the chemocline. The monimolimnion is rich in phosphorus, nitrogen and sulphide creating an environment suitable for bacterial growth, and exceptional preservation due to lack of mixing (Fry 1986).

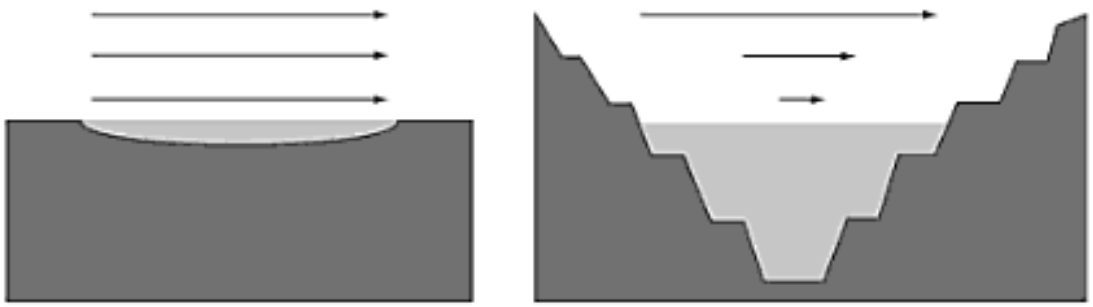

Figure 1.19: Typical lake morphology (left) allows wind energy to mix water, particularly if the lake has a large surface area and shallow waters. This allows for turnover to occur at least once a year. Steep geometry, small surface area and large depth prevent wind energy from turning over water (right). This promotes stratification and meromixis (from Castendyk 2009).

In order to determine the affinity for meromixis (and thus preservation potential) in lakes, a calculation based on the relationship between surface area (SA) and depth was developed by Hutchinson (1957),

$$Zr = \frac{(50 \times Zm \times \sqrt{\pi})}{(\sqrt{SA})} \quad (1)$$

where Zr= relative depth, *Zm*= maximum depth, *A*= surface area

Where, lakes with a *Zr* of 2-45% were considered meromictic (Castendyk, 2009).

Castendyk (2009) notes that the value of *Zr* as a tool is limited when attempting to predict past and future limnology. For example, some pit lakes do not show a consistent relationship between *Zr* and meromixis. Lake Goitsche in central Germany (*Zr*=1%) exhibits permanent stratification (Boehrer et al. 2003) whereas Blowout lake in Utah (*Zr*= 34%) undergoes episodes of complete turnover (Castendyk and Jewell 2002).

Using data from Hubeny et al. (in press), the affinity for meromixis was calculated using Hutchinson's equation for Sluice Pond as its geometry varied in response to climate-driven water level change.

Table 1.3: Date and approximate affinity for meromixis (*Zr*) based on relative depth of Sluice Pond where a *Zr* of 2-45% generally describes a meromictic lake. A low affinity for meromixis was found during the Middle Holocene drought, where *Zr*= 1.7.

Date (cal BP)	**Metres below lake level (m bmll)**	**Surface area (m^2)**	**Relative depth (*Zr*)**
11.5	~20.0	15,700	2.12
7.9	~15.3	84,000	2.7
6.8	~6.0-12.0	220,000-174,000	1.1-2.6
4.3	~11.2-14.7	141,000-84,000	2.5-2.2
4.3-0	~13.7-0	140,000-180,000	1.7-5.2

1.5.5 Objectives of this study

The main aim for this thesis was to further our understanding of Sluice Pond following the in-depth research of Hubeny et al. (in press) using dinoflagellates, thecamoebians and ciliates. The objectives of this study were:

1) Identify the main environmental controls on dinocyst and protozoan distribution in Sluice Pond;
2) Investigate the effects of climate variability and anthropogenic activity on algal and protozoan communities since the early Holocene;
3) Identify new and unknown dinoflagellate cysts.

The results of studies from the early Holocene to anthropogenic activity are in Chapter 2 while studies of surface samples and anthropogenically impacted cores are present in Chapter 3.

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Chapter 2: Natural and cultural eutrophication of Sluice Pond, MA recorded by algal and protozoan microfossils

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2.1 Introduction

This paper examines the response of dinoflagellates and agglutinated protozoans (thecamoebians and ciliates) to natural and anthropogenic change in Sluice Pond since deglaciation. This small (~18 hectares), deep (20 m) partially meromictic lake (Figure 2.1) in the City of Lynn, Massachusetts is surrounded by two major roads, a housing development and a golf course on the southwest portion of the lake. It is a popular recreational area where the public can access swimming and fishing (City of Lynn 2012). Lynn was first settled in 1629 and incorporated in 1850 due to a boom in leather tanning production and shoe making (Herbert 1890). This city whose current population is 91,000 (United States Census 2010) is primarily a bedroom community for nearby Boston.

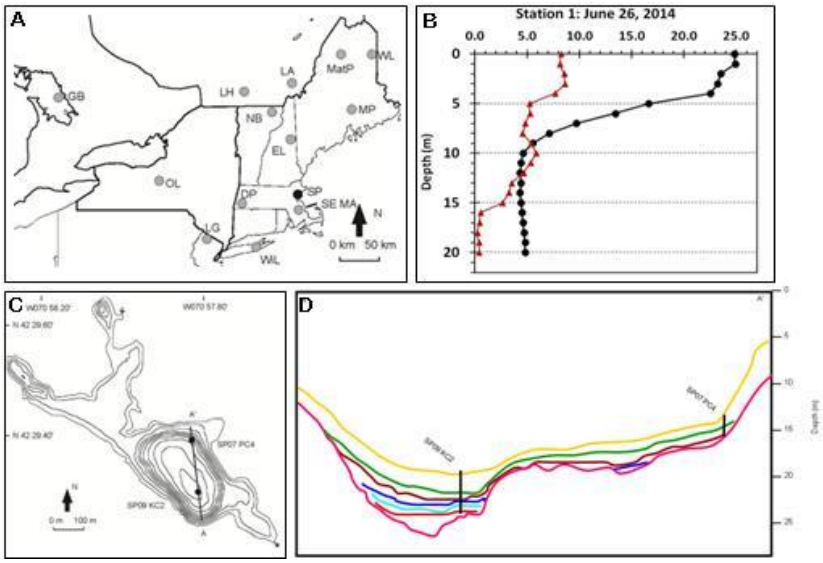

Figure 2.1: a. The location of Sluice Pond in eastern Massachusetts is shown by the black dot. b. The deep basin of Sluice Pond is permanently stratified, with a sharp thermocline measured on June 26, 2014 below 4 m and anoxic conditions below 15 m. c. Sluice Pond, showing the deep basin (*Zmax* ~ 20 m; bathymetric interval 2m) and locations of the basin (SP09 KC2) and margin (SP07 PC4) cores. d. Cross-section from A-A' across Sluice Pond with seismic reflectors (modified from Hubeny et al. in press). The yellow seismic reflector represents the lake bed.

Dinoflagellates are unicellular algae that belong to the Phylum Dinoflagellata Bütschli, Class Dinophyceae Pascher. They are an important part of the summer phytoplankton in North American lakes, second only to diatoms (Wehr and Sheath 2003). Dinoflagellates reach optimal growth during times of high illumination, nutrient levels and temperature (Taylor 1987), and some taxa form fossilizable resting cysts when conditions become sub-optimal. Cysts of freshwater dinoflagellates have been found in palynological preparations in sediments as old as Oligocene (Traverse 1955) and are quite common in Holocene sediments (Norris and McAndrews 1970; Boyko 1973; Boyko- Diakonow 1979; Dale et al. 1999; Tardio et al. 2006a, b; Chu et al. 2008; Danesh et al. 2013; McCarthy and Krueger 2013) but they have mostly been ignored in paleolimnological studies. This is largely due to their poorly understood taxonomy, with resting cysts described for only 84 of the ~350 dinoflagellate species known from freshwater environments (Mertens et al. 2012). Cysts attributed to the genera *Peridinium* Ehrenberg and *Parvodinium* Carty have recently been shown to reflect natural and anthropogenic change (principally eutrophication) in the water column of eastern North American lakes (Burden et al. 1985; Zippi et al. 1990, 1991; McCarthy et al. 2011; Krueger 2012; McCarthy and Krueger 2013; Danesh et al. 2013; Volik 2014), illustrating their potential as paleolimnological proxies.

Thecamoebians (also referred to as testate amoebae) are protozoans found in freshwater environments such as lakes, rivers, ponds, and sufficiently moist habitats such as wetlands, bogs and moderately saline environments such as estuaries (Ogden and Hedley 1980; Medioli and Scott 1988; Beyens and Meisterfeld 2002). Thecamoebians belong to Class Rhizopoda von Siebold, Order Arcellinida Kent, and Superfamily Arcellacea Ehrenberg, and thus are sometimes referred to as arcellaceans. Most common thecamoebians in North American lakes belong to the Family Centropyxidae Jung or Difflugiidae Stein. These families are characterized by fossilizable tests made of organic material, similar to chitin, that are agglutinated with detrital particles including silt and diatoms (Medioli and Scott 1987, 1988), with difflugiid taxa being more coarsely agglutinated. Thecamoebians feed on bacteria, algae, fungi or other protozoa (Ogden and Hedley 1980) and are an important part of the benthos in North American lakes (Collins et al. 1990; Neville et al. 2010a). Thecamoebians have been reported from Cretaceous rocks from the Western Canadian Foreland Basin, from Carboniferous coal measures in Cape Breton Island and from the Neoproterozoic Chuar Group, Grand Canyon (Thibaudeau and

Medioli 1986a, b; Medioli et al. 1990a, b; Wightman et al. 1994; Porter and Knoll 2000). They have been used as indicators of climate, pH, heavy metal toxicity, salinity, dissolved oxygen (DO) and temperature in addition to eutrophication, (McCarthy et al. 1995; Reinhardt et al. 1998; Kumar and Patterson 1999; Patterson and Kumar 2002; Neville et al. 2010, 2011; Patterson et al. 2012; Danesh et al. 2013).

Like thecamoebians (with which they are sometimes confused) ciliates can be found in rivers, lakes, ponds, wetlands and other sufficiently moist habitats. Ciliates are a group of protozoans that belong to Class Spirotrichea Small and Lynn, Order Tintinnida Kofoid and Campbell, Family Codonellidae Kent. They are characterized by hair-like organelles called 'cilia', which are used for swimming, crawling and feeding (Foissner and Wilbert 1979). The presence of ciliates such as *Codonella cratera* Leidy in sediments has been observed in areas of increased eutrophication and anoxic, muddy bottom waters (Barbieri and Orlandi 1989; Danesh et al. 2013; Volik 2014).

2.2 Materials and methods

A 4.72 m long core (SP09 KC2) was taken in September 2009 from the deepest part of Sluice Pond using a modified Kullenberg-type gravity piston corer, and a 2.02 m long core (SP07 PC4) was taken in September 2007 from shallower water using a rod-driven piston corer (Figure 2.1). Global positioning system (GPS) determined the coring locations to be 42°29.300' N x 070°57.815' W for core SP09 KC2 and 42°29.393' N x 070° 57.831' W for SP07 PC4 on the basin margin, with water depths determined to be 19.7 and 13.3m, respectively, using standard "fish finder" SONAR. Sub-bottom seismic reflection data were collected using a Stratabox sub-bottom system (10 kHz) with coupled Garmin GPS unit along transect A-A' in 2009 and 2010 (Hubeny et al., in press) and water quality parameters (temperature, pH, conductivity and DO) were measured using a YSI multiprobe on June 26, 2014 (Figure 2.1).

Good chronological control is available based on six ^{14}C ages of terrestrial macrofossils (calibrated using Clam) and two ^{210}Pb and ^{137}Cs ages, augmented with the well-dated pollen zonation for southern New England (Foster and Zebryk 1993, Newby et al. 2000, Shuman et al. 2001, 2004, Webb et al. 2004) (Hubeny et al. in press). The age model for the deep basin core, which exhibits apparently constant and continuous sedimentation over the last ~11,400 years, was calculated by Hubeny et al. (in press) using the following equation:

$$y = 2e^{-7x^4} - 0.0002x^3 + 0.0614x^2 + 18.852x - 35 \quad (2)$$

Where y = age in calibrated years before present, and x = depth in core (cm).

Calculated ages for core SP09 KC2 are shown in the appendices, together with absolute abundances (concentrations) and relative abundances (percentages) of dinocysts and of common protozoan microfossils. The chronology of core SP07 PC4 is less well constrained and is associated with a hiatus spanning at least 5,400 – 3,500 cal BP (the hemlock minimum; see Hubeny et al. in press).

The cores were sub-sampled at the Geology Department in Salem State University and processed and analyzed for pollen (see Hubeny et al. in press) and aquatic microfossils at Brock University. Sub-samples of 2.5-ml volume (measured by liquid displacement) were prepared for palynological analysis in a hot bath (60°C) using aprocess modified from Faegri and Iversen (1975) to allow dinoflagellates to be analyzed (McCarthy et al., 2011). The sediments were disaggregated using a weak solution (0.02%) of sodium hexametaphosphate. Carbonates were dissolved using 10% hydrochloric acid (HCl) with a tablet containing 10, 850 ± 200 (batch # 006720) *Lycopodium clavatum* marker spores to quantify total palynomorph concentrations (Stockmarr 1971), and with 48% hydrofluoric acid (HF) to dissolve silicates and diatoms. The samples were centrifuged at 3000 rpm for 3 minutes and washed with distilled water after each step. Samples were sieved with a 10-μm *Nitex* mesh and mounted on slides using glycerin jelly. Cysts were counted and identified at 400 X following McCarthy et al. (2011) and Krueger (2012). Except in dilute core-top sediments and core-bottom sediments, statistical validity was achieved following Patterson and Fishbein (1989) who demonstrated that minimum counts of 50 specimens are valid if >50% of the assemblage is dominated by one species.

One to ten-ml sub-samples were sieved using a three-tiered sieve (500-μm, 63-μm and 45-μm) for thecamoebian and ciliate analysis. The 500-μm sieve was used to capture large debris. Most specimens of larger thecamoebian taxa such as *Centropyxis aculeata* Ehrenberg, *Difflugia oblonga* Ehrenberg and *Difflugia corona* Wallich were retained in the 63 to 500-μm fraction, but the majority of specimens of *Cucurbitella tricuspis* (Carter) Medioli et al. and *Difflugia protaeiformis* Lamarck and of the tintinnid ciliate *Codonella cratera* were captured by the 45-μm sieve. After sieving, samples were analyzed wet in a Petri dish using a Leica MZ 125 microscope. Minimum counts of 200 tests were achieved in nearly all samples deposited since

~5,000 years ago except in soupy core-top sediments, with most counts exceeding 300 cysts ensuring statistical validity (Patterson and Fishbein 1989). Thecamoebians were identified using the key of Kumar and Dalby (1998) and ciliates were identified using SEM images in Beyens and Meisterfeld (2002).

Aquatic microfossils are plotted as absolute abundances (concentrations/ ml) against calibrated age; raw counts, relative abundances, error of estimate and SDI calculations are available in the appendices. Species diversity was calculated using the Shannon Weaver Diversity Index (SDI) (Shannon and Weaver 1949) as follows:

$$SDI = -\sum_{I}^{S}(F/N) \times \ln(F/N) \quad (3)$$

F= relative abundance and *N*= number of specimens

Data analysis software (PAST) developed by Hammer et al. (2001) was used to statistically analyze the relatedness of aquatic microfossil in samples using constrained cluster analysis.

2.3 Results

2.3.1 Core SP09 KC2

Nine cyst morphotypes were distinguished in Sluice Pond sediments, four of which are common to abundant in most samples (Photo plate 2.1). The affinities of two of the common morphotypes are known through germination studies and LSU/SSU rDNA-based phylogenies (Wall and Dale 1968; McCarthy et al. 2011, 2013): *Peridinium willei* Huitfeld Kaas, and *Peridinium wisconsinense* Eddy. A third cyst is referred to as *Peridinium* cf. *volzii* based on spontaneous excystment of *Peridinium volzii* Lemmermann studies from Crawford Lake (Krueger 2012). Large, ornate cysts are tentatively attributed to *Peridinium gatunense* Nygaard by comparison with photographs of a cyst of this taxon in Pfeister (1977) and Viner-Mozzini et al. (2003), but this needs to be confirmed through germination or DNA studies. Except for distinctive cysts of *Peridinium limbatum* (Stokes) Lemmermann (Wall and Dale 1968; Norris and McAndrews 1970), the affinities of rare cyst morphotypes in the samples are unknown.

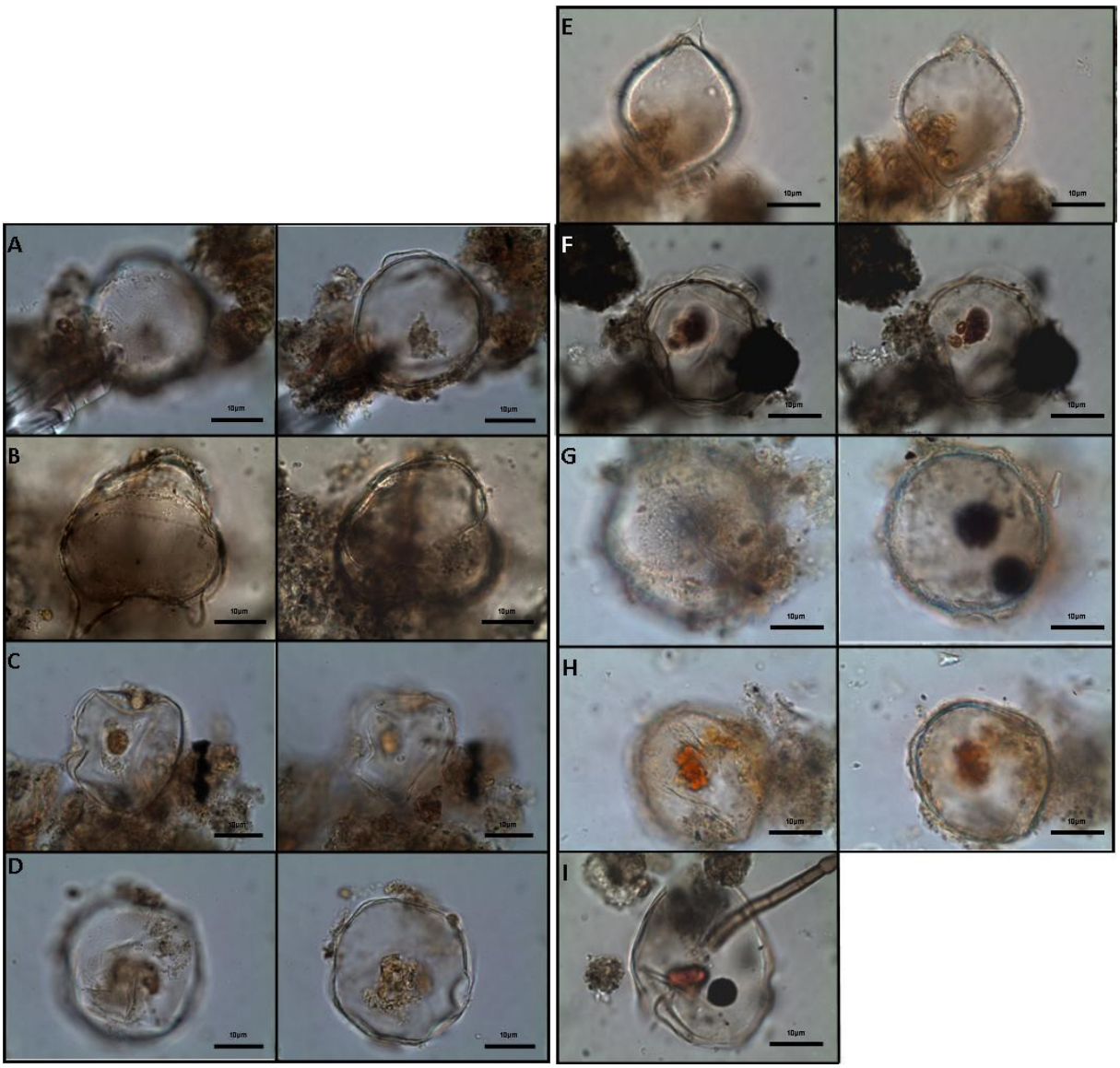

Photo plate 2.1: Photomicrographs of dinocysts in Sluice Pond. A) *Peridinium* cf *gatunense* Nygaard. B) *Peridinium limbatum* (Stokes) Lemmerman. Cysts of *P. limbatum* are described as large (92 - 104 x 64 - 82 µm), roughly pentagonal cysts with one apical and two antapical projections. The archeopyle is a lateral suture and the wall texture is granular (Mertens et al. 2012). Cysts in this study also display visible paracingulum and paratabulation. C) *Peridinium volzii* Lemmerman. D) *Peridinium willei* Huitfeld-Kaas. E) *Peridinium wisconsinense* Eddy. Additional photoplates and indepth cyst descriptions are available in Photoplate 6.1.

The following cysts lack formal names are described herein as cyst types. Germination is required for further classification. F) Cyst type G is a medium sized (~30 µm), cavate (epicavate), proximate cyst, easily identified by its rounded shape, and thin, continuous

ectophragm. G) Cyst type J is a large (longitudinal length: ~40 – 45 µm), cavate, proximate cyst with a visible paracingulum, thick and continuous ectrophragm and dark-brown colouration, H) Cyst type K is smaller (longitudinal length: ~25 µm) than Cyst type J, however, it displays a similar appearance. I) Cyst type L is a medium sized (~35 µm), ovoid, cavate, proximate cyst with a clear paracingulum that is visible in dorsal and ventral view.

Dinoflagellate cysts range from ~2,700 to 63,201 cysts/ml, with the lowest concentration in soupy sediments at the top of the core (Figure Figure 2.2). Cysts of *Peridinium* cf. *gatunense* are especially common (7-78 % of cysts) in sediments in low to mid-diversity assemblages (SDI=0.5 – 1.4) deposited in this deep basin over the last ~5,000 years, and their occurrence usually correlates with *P. willei* and *P.* cf. *volzii*, but inversely with *P. wisconsinense* (Figure 2.2). *P. wisconsinense* generally dominates cyst assemblages prior to 5,000 cal BP (especially since 9,500 cal BP) where they make up an average of approximately of 63% of low-diversity assemblages, ranging from 21 to 86 % (SDI= 0.6 -1.4).

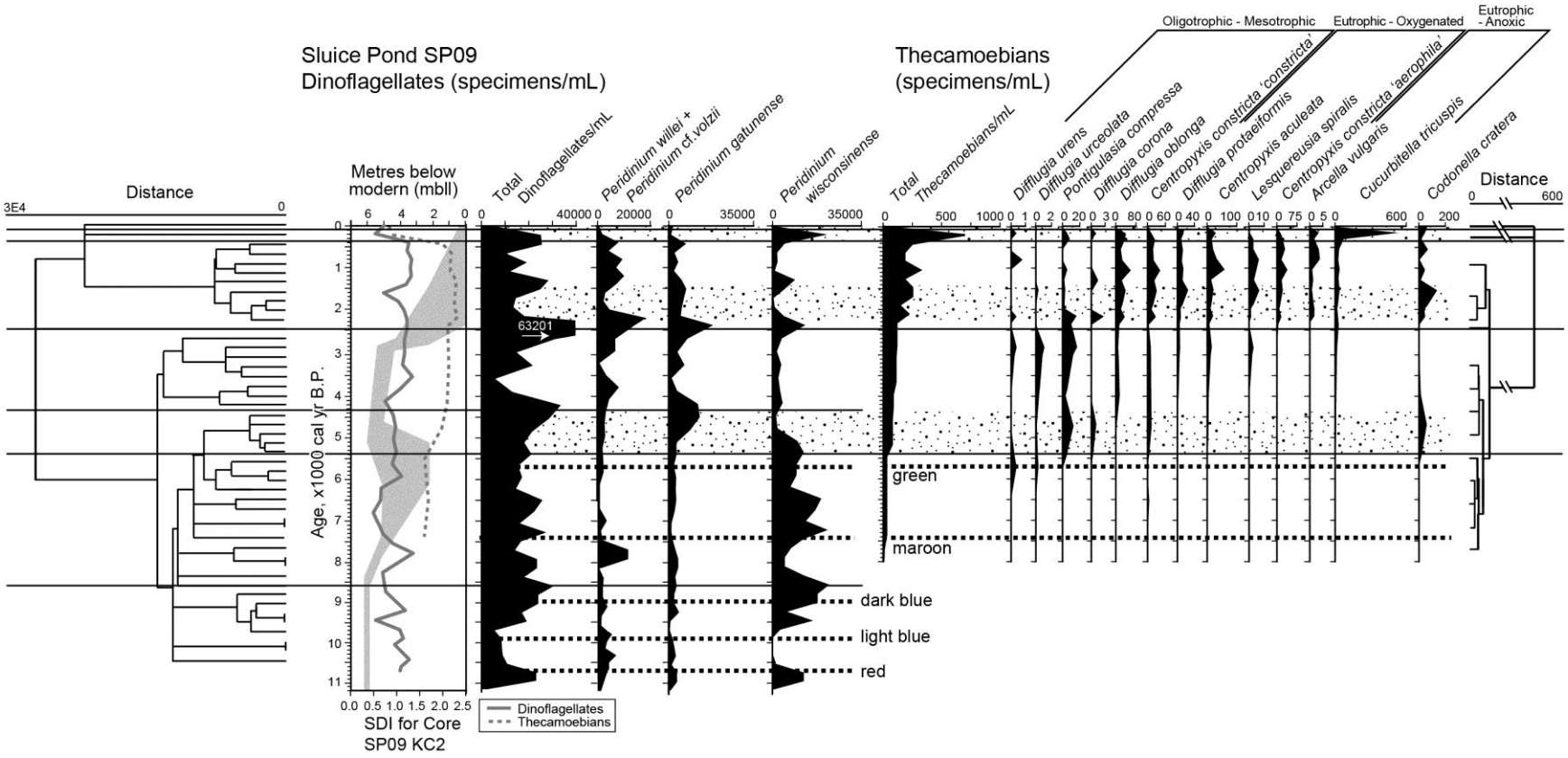
Sluice Pond SP09
Dinoflagellates (specimens/mL)
Thecamoebians
(specimens/mL)
Oligotrophic - Mesotrophic
Eutrophic - Oxygenated
Eutrophic - Anoxic
Distance
Metres below modern (mbll)
Total Dinoflagellates/mL
Peridinium willei + Peridinium cf. volzii
Peridinium gatunense
Peridinium wisconsinense
Total Thecamoebians/mL
Difflugia urens
Difflugia urceolata
Pontigulasia compressa
Difflugia corona
Difflugia oblonga
Centropyxis constricta 'constricta'
Difflugia protaeiformis
Centropyxis aculeata
Lesquereusia spiralis
Centropyxis constricta 'aerophila'
Arcella vulgaris
Cucurbitella tricuspis
Codonella cratera
Distance
63201
Age, x1000 cal yr B.P.
green
maroon
dark blue
light blue
red
SDI for Core SP09 KC2
Dinoflagellates
Thecamoebians

Figure 2.2: Microfossils in core SP09 KC2 from the deepest basin in Sluice Pond plotted against years BP based on five radiocarbon ages and four pollen zone boundaries (see age model in Hubeny et al. in press). Also shown are published lake level reconstructions from New England (Webb et al. 2004) and Shannon Diversity Index (SDI) for both microfossil groups, and stippling identifies oxygen depleted zones. Constrained cluster analysis identifies three major assemblage changes in aquatic microfossils ~5,000 cal yr BP in response to a rapid decline in lake level driven by the mid-Holocene drought that produced the hemlock "crash", ~2,200 cal yr BP in response to continued eutrophication and fluctuating lake level, and 300 years ago in response to anthropogenic impact.

Thecamoebian tests are absent below the maroon reflector dated ca. 7,400 cal BP (Hubeny et al. in press) and they are sparse and statistically insignificant (< 25 tests/ml, mainly the ubiquitous *Difflugia oblonga* and *Centropyxis constricta* Ehrenberg) until above the green reflector (Figure 2.2). A relatively diverse fauna rich in *Pontigulasia compressa* Carter and *Difflugia urceolata* Carter increase in abundance above the green reflector (c. 5,700 cal BP), and *Arcella vulgaris* Ehrenberg, *Lesquereusia spiralis* Ehrenberg, *Difflugia protaeiformis*, and *Centropyxis aculeata* increase sharply in abundance ~2,200 years ago where there is a peak in abundance of the ciliate *Codonella cratera*. There is a marked increase in *Cucurbitella tricuspis* in sediments rich in ragweed and other non-arboreal (weedy) pollen deposited over the last c. 300 years (Hubeny et al. in press), but thecamoebian concentrations are sparse at the surface (~8 thecae/ ml) where the most common protozoan is the ciliate *C. cratera*.

2.3.2 Core SP07 PC4

The absence of seismic reflectors between the pink glacio-marine unit and the maroon reflector records a hiatus through the early Holocene at the marginal site. Lacustrine sediments at the base of core SP07 PC4 were dated ~7,800 cal BP, but pollen analysis identified another hiatus spanning the hemlock minimum above the green reflector corresponding to the mid-Holocene drought. (in press). Similar dinocyst assemblages were found in the marginal core, but species diversity lower (ranging from SDI= 0.5-1.3, with only seven cyst types found) and *Peridinium* cf. *gatunense* is much less abundant (peaking around 33% compared with 78% in the deep basin core) (Figure 2.3). Concentrations are also generally lower, ranging from 1,436 to 9,750 cysts/ml, except in ragweed pollen–rich sediments where they rise sharply to 264,089 cysts/ml before declining again to 2,750 cysts/ml at the top of the core. *P. wisconsinense* dominates the assemblage until the unconformity spanning ~5,400-3,500 cal BP. Above the unconformity, *P.*

willei and *P.* cf. *volzii* co-dominate in sparse assemblages until just below the surface, when a diverse assemblage very rich in *P. wisconsinense, P.* cf. *gatunense, P. willei* and *P.* cf. *volzii* was found in ragweed pollen-rich sediments.

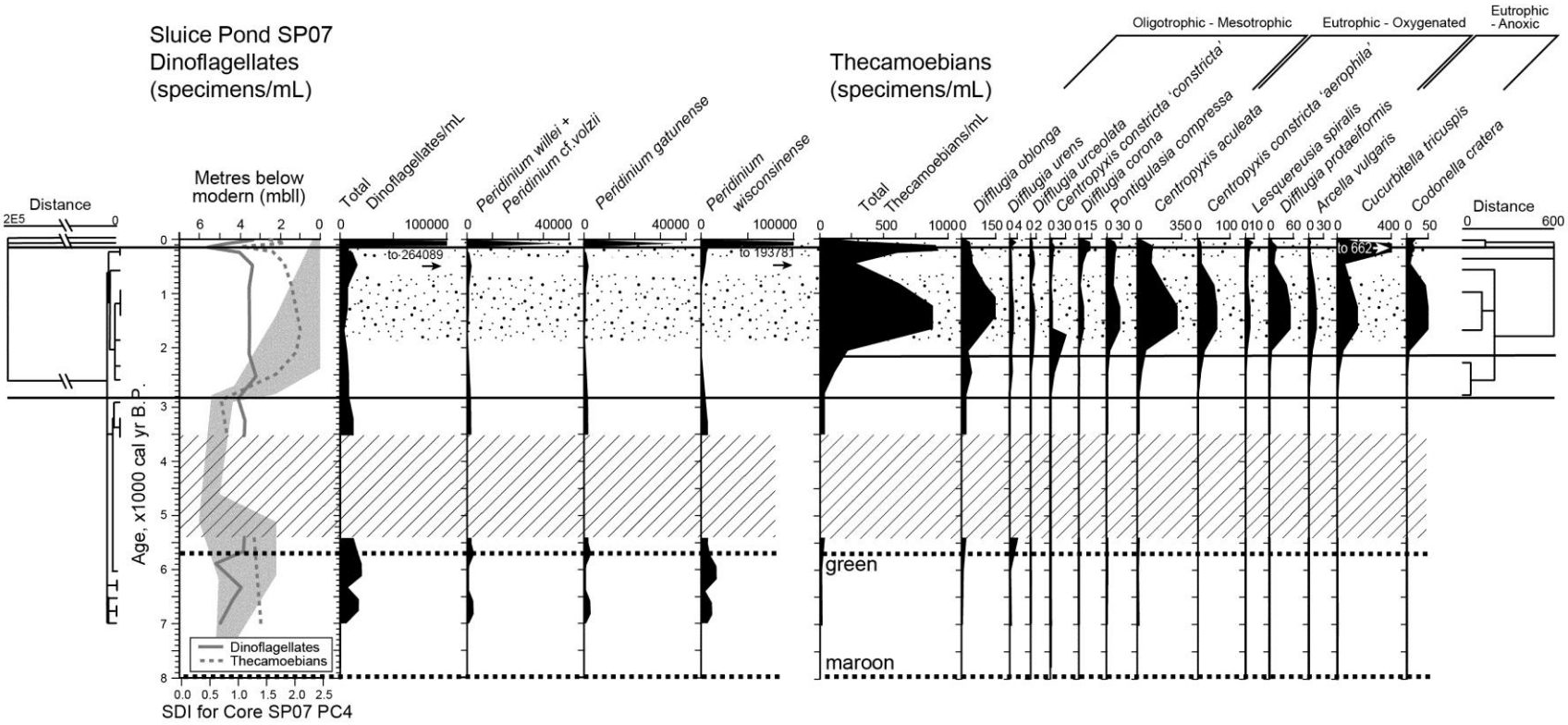
Sluice Pond SP07
Dinoflagellates
(specimens/mL)
Thecamoebians
(specimens/mL)
Oligotrophic - Mesotrophic
Eutrophic - Oxygenated
Eutrophic - Anoxic
Distance
Metres below modern (mbll)
Total Dinoflagellates/mL
Peridinium willei + Peridinium cf.volzii
Peridinium gatunense
Peridinium wisconsinense
Total Thecamoebians/mL
Difflugia oblonga
Difflugia urens
Difflugia urceolata
Centropyxis constricta 'constricta'
Difflugia corona
Pontigulasia compressa
Centropyxis aculeata
Centropyxis constricta 'aerophila'
Lesquereusia spiralis
Difflugia protaeiformis
Arcella vulgaris
Cucurbitella tricuspis
Codonella cratera
to 264089
to 193781
to 662
Age, x1000 cal yr B.P.
green
maroon
Dinoflagellates
Thecamoebians
SDI for Core SP07 PC4

Figure 2.3: Microfossils in core SP07 PC4 from 13.3m water depth plotted against years BP with hatches showing the hiatus spanning the hemlock minimum (5,400 – 3,500 cal yBP) and assuming cocntinuous and constant sedmimentation thereafter. Also shown are the lake level reconstructions ofWebb et al. (2004) for New England and Shannon Diversity Index (SDI) for both microfossil groups, and stippling identifies oxygen depleted zones. Cluster analysis identified major assemblage breaks in dinoflagellate cysts and protozoans at the same times as in the basin core: ~5,000, 2,200 and 300 years ago.

Very sparse, low-diversity populations of thecamoebians, mainly *Centropyxis aculeata,* are present in sediments deposited belowthe green reflector (Figure 2.3). A similar assemblage was established above the erosional unconformity until ~2,200 years ago, when a rich assemblage containing *Pontigulasia compressa, Difflugia urceolata, D. protaeiformis, D. oblonga, Centropyxis constricta* 'constricta' and the ciliate *Codonella cratera* was established. A marked increase in thecamoebian concentrations in sediments rich in ragweed and other non-arboreal (weedy) pollen deposited over the last few centuries is almost entirely comprised of *Cucurbitella tricuspis*. As in core SP09 KC2, thecamoebian concentrations are sparse at the surface (~17 thecae/ml) and the most common protozoan is *C. cratera* (~18 loricae/ml).

Discussion

Sediments from Sluice Pond hold an extraordinary record of cysts of freshwater dinoflagellates. Dinocyst concentrations are very high compared with other paleolimnological studies such as Crawford Lake where total dinocyst concentrations range from <1,000- 45,000 cysts/ml and Cook's Bay where ~1,000- 12,000 cysts/ml were identified (Danesh et al. 2013; McCarthy and Krueger 2013). In contrast, other non-pollen palynomorphs (e.g. *Pediastrum*, desmids, etc.) are relatively sparse in Sluice Pond compared to other sites, such as Crawford Lake (McCarthy and Krueger 2013), Lake Simcoe (Danesh et al. 2013; Volik 2014) and small lakes in the Penetanguishene region of Ontario (Burden et al. 1986). Dinocyst species diversity is also unusually high: at least nine morphotypes were present in the basin core SP09 KC2 (Figure 2.2), seven of which were also found in the margin core SP07 PC4 (Figure 2.3). Assemblages of freshwater dinocysts are commonly monospecific, or nearly so (Batten et al. 1999; Tardio et al. 2006a, b; Chu et al. 2008) and Holocene palynological studies from eastern North American lakes identified a maximum of four morphotypes (Norris and McAndrews 1970; Burden et al.

1986; Zippi et al. 1990, 1991; McCarthy et al. 2011; Danesh et al. 2013; McCarthy and Krueger 2013; Volik 2014). Moreover, cysts attributed to *Peridinium gatunense* that are common to abundant in sediments from Sluice Pond have not been reported from previous palynological studies, although limnological studies from Lake Kinneret, Israel report the generation of resting cysts of *P. gatunense* during massive blooms that were common until the mid-1990s when hydrological modifications prevented the Jordan River from flowing into Lake Kinneret (Sea of Galilee) (Zohary et al. 2012), and laboratory testing supported the hypothesis that nutrient-enriched water from the Jordan River caused *P. gatunense* to bloom (Zohary et al. 2001). The distinctive morphology of these large (longitudinal length 40 – 45 μm) cysts, with clear paratabulation, a distinct paracingulum and a discontinuous ectophragm (Figure 2), makes it unlikely that they would be missed or misidentified by palynologists, so we assume that it was not present in the small number of studies that attempted to identify the cysts of freshwater dinoflagellates to species.

The relative scarcity of large, ornate cysts attributed to *P. gatunense* in the marginal core suggests that its preservation is favored by the low bottom water oxygen concentrations in the deep basin, possibly explaining the absence of this cyst in the palynological literature. Elevated dinoflagellate populations reflect blooms in response to nutrient flux (Popovsky 1983; Carty 2002) but this is only translated into high dinocyst concentrations in sediment if taphonomic conditions promote preservation. The exceptional dinocyst record of Sluice Pond is attributed in large part to enhanced preservation, particularly in the deep basin core (Figure 2.2) where dinocysts concentrations average ~19,500 cysts/ml (range ~2,700 – 63,000 cysts/ml) (Appendix 1d). Low dissolved oxygen concentrations resulting from stratification has been found to promote the preservation of dinocysts in other meromictic lakes like Crawford Lake (Krueger 2012; McCarthy and Krueger 2013), and anoxia resulting from increased biochemical oxygen demand (BOD) due to cultural eutrophication promoted dinocyst preservation even in holomictic lakes like Georgian Bay and Lake Simcoe (McCarthy et al. 2011; Danesh et al. 2013; Volik 2014). The much lower concentrations of dinocysts prior to anthropogenic impact and cultural eutrophication (average ~3,800 cysts/ml, range ~1,400 – 9,750 cysts/ml) and the abundance of *P.* cf. *gatunense* in ragweed-rich sediments at the top of marginal core are consistent with this interpretation.

Waters in the deep basin of Sluice Pond are currently anoxic (Figure 2.1), and the affinity for meromixis was found to be high in this small, deep basin through most of the postglacial, calculated using an equation developed by Hutchinson (1957) based on the relationship between surface area and depth:

$$Zr = \frac{(50 \times Zm \times \sqrt{\pi})}{(\sqrt{SA})} \quad (4)$$

Zr= relative depth, *Zm*= maximum depth, *SA*= surface area

According to Hutchinson's equation *Zr* was ~ 2.12% during the arid early Holocene (~ 11.4 – 8.2 ka) when its surface was ~15.0-20.0 m below modern lake level/bmll, based on the absence of the red, light blue and dark blue seismic reflectors in depths shallower than 23.5 m bmll (Hubeny et al. in press) and the SA was 15,708-m^2 (based on the rough ellipse shape of the basin and the seismic stratigraphy (Figure 2.1). Climatic reconstructions using pollen-derived transfer functions prior to ~ 8,200 years ago in core SP09 KC2 (Figure 2.4) confirm that hydrological conditions were drier than today, although they, together with the several reflectors, suggests greater hydroclimatic variability than lake level reconstruction of Webb et al. (2004) implies (Figures 2.2, 2.3). The confinement of seismic reflectors to the deep basin of Sluice Pond confirms was thus a very small (~3 m deep), steep-walled isolated, hydrologically closed and stratified pond during the early Holocene, and the resulting bottom water anoxia is consistent with low $\delta^{34}S$ values (Figure 2.4) and with the absence of thecamoebians (Figure 2.2). These benthic protozoans have been found even in hyper-oligotrophic lacustrine environments (McCarthy et al. 1995; Neville et al. 2010) and in ecosystems severely impacted by tailings and effluent from both metal mining and oil sands mining operations (Patterson et al. 1996, 2012; Reinhardt et al. 1998; Patterson and Kumar 2002; Kihlman and Kauppila 2009; Neville et al. 2011), and there is no evidence that the deep basin of Sluice Pond dried up, which Sonnenburg et al. (2013) suggested for the absence of these normally ubiquitous microfossils from early Holocene sediments in Rice Lake, Ontario.

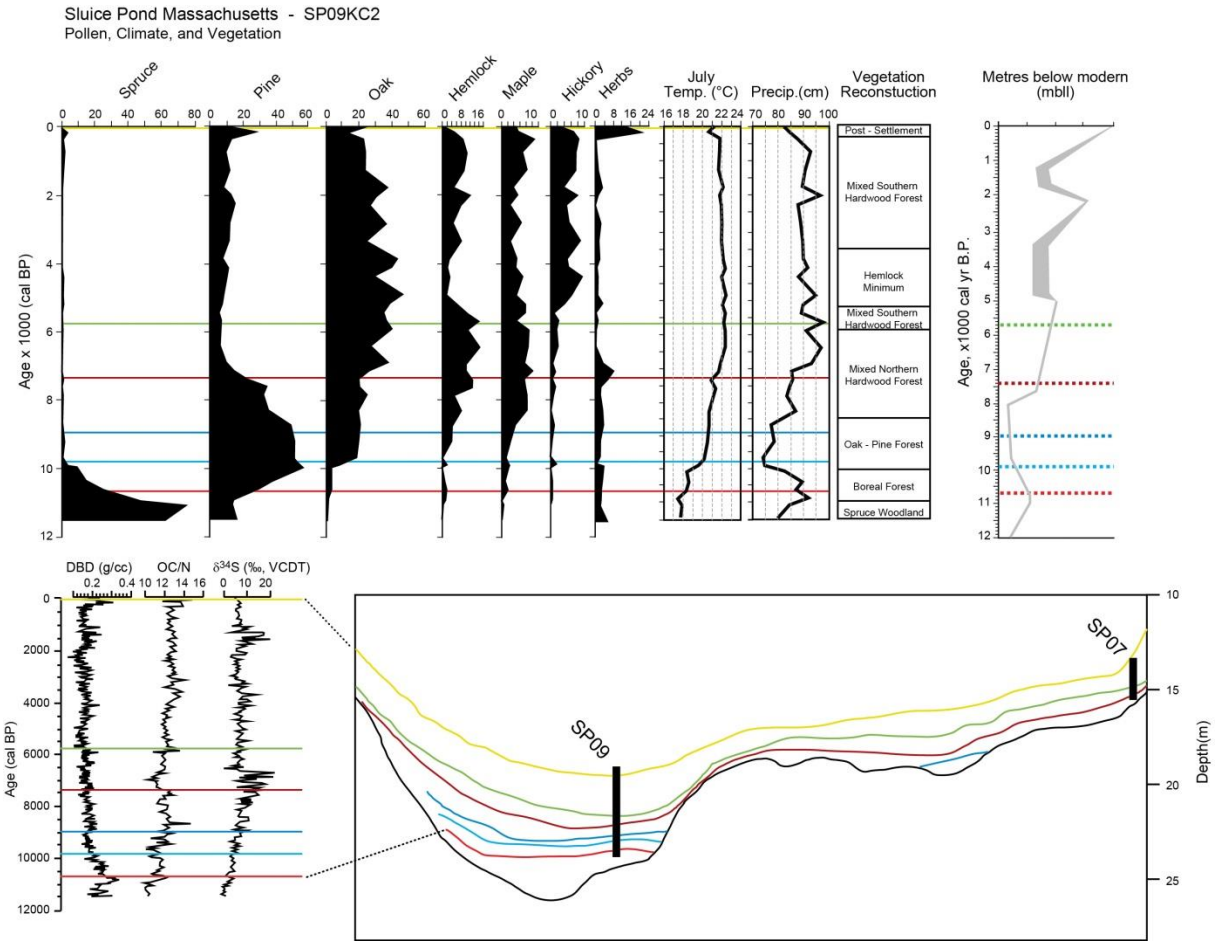

Figure 2.4: Major changes in phytoplankton (dinoflagellate), zooplankton (ciliate) and zoobenthos (thecamoebians) assemblages in Sluice Pond correlate with pollen-derived reconstructions of vegetation and climate and stable isotope and lithostratigraphic proxies of climate-driven lake level change since the early Holocene (Hubeny et al. in press), allowing us to refine the lake level reconstruction of Webb et al. (2004) to show slightly more hydroclimatic variability during the early Holocene and a late Holocene lowstand correlating with lowstands in Lakes Erie and Ontario.

Thecamoebians failed to colonize Sluice Pond throughout the early Holocene (as defined by Walker et al. 2012) despite evidence of increasing lacustrine productivity recorded not only by abundant dinocysts but also by a rise in organic carbon (OC) concentrations from a low of ~6.18% at the base of the core to 14.13% by ~9ka in core SP09 KC2, as well as a trend toward less negative $\delta^{13}C$ values (Hubeny et al. in press). Increases in dinocyst species diversity (SDI) associated with seismic reflectors (Figure 2.2) suggest increasingly hospitable conditions attributed to an increase in nutrient concentrations during early Holocene lowstands. Rising lake levels during the intervening wetter intervals during the early Holocene, in contrast, saw a strong

dominance of cysts of *Peridinium wisconsinense* which is interpreted as recording low nutrient availability in the water column. This endemic North American taxon is typically common in oligotrophic – mesotrophic waters with relatively high pH but other taxa, such as *Peridinium willei*, tend to be more common as part of a more diverse flora in eutrophic, low pH environments (Zippi et al. 1990, 1991; McCarthy et al. 2011).

The marginal core site (SP07 PC4, ~15.3 m bmll) was initially transgressed following the lowstand that produced the maroon reflector (Figure 2.3) in response to wetter conditions recorded by pollen and geochemical data (Hubeny et al. in press). Lake levels rose throughout New England (Figure 2.2, 2.3) as pine-dominated boreal woodland/ boreal forest vegetation was succeeded by a mesic mixed forest characterized by maple, beech and hemlock (Shuman et al. 2001; Webb et al. 2004). Transgression of the marginal core site is consistent with an increase in precipitation of nearly 10 cm/year between 8.7 and 8.3 ka (Hubeny et al. in press). The rise in $\delta^{34}S$ in the deep basin core (Figure 2.4) records greater mixing of the water column and oxygenation of bottom waters early in the transgression, allowing the establishment of a sparse, low-diversity thecamoebian fauna on the margin and in the basin (Figures 2.2, 2.3). Dilution of nutrients by the influx of water is recorded by the strong dominance of cysts of *Peridinium wisconsinense* during this interval (Figures 2.2, 2.3). Values of $\delta^{34}S$ values declined sharply ~ 6,700 years ago, suggesting that the threshold depth for lake stratification (estimated at ~14 - 15.5 m bmll; Hubeny et al. in press) was reached as transgression increased the surface area to ~84,000 m^2, consistent with peak warmth and moisture during the middle Holocene hypsithermal (Figure 2.4).

A sharp decline in lake level accompanied the warm, dry conditions that produced the hemlock "crash" decline throughout eastern North America that spans ~5,300 – 3,200 years ago in New England (Newby et al. 2000; Shuman et al. 2001; Webb et al. 2004). Lake level was ~11.2 -14.7 m bmll, which is consistent with the depth at which the green reflector pinches out (Figure 2.4) and with the estimated decrease in lake levels of 4 - 6 m bmll in Crooked Pond during the hemlock minimum (Shuman et al. 2001). Abundant macrophyte remains observed in the basin core site suggest that lake levels fell markedly during this interval (Hubeny et al. in press). The hiatus in the margin core spanning the entire hemlock minimum zone (Figures 2.2, 2.3) attests to the mid-Holocene aridity in New England coeval with the global signal for aridity 4.2 ka from proxy data (foraminifera, thecamoebians, stable isotopes, dune systems, etc.)

recording global aridity suggested by Walker et al. (2012) as the boundary between the middle and late Holocene Epoch. This is consistent with the theory that the decline in hemlock, evident in pollen diagrams throughout eastern North America at mid latitudes, was driven by drought (Webb 1988; Newby et al. 2000; Shuman et al. 2004) as opposed to pathogens (Bhiry and Filion 1996).

A sharp change in dinocyst flora in the basin core, from a low-diversity *Peridinium wisconsinense*- dominated flora to a more diverse *Peridinium* cf. *gatunense*- dominated flora (Figure 2.2) is interpreted as a response to increased nutrient concentration during the mid-Holocene lowstand. Although *P.* cf. *gatunense* has not previously been reported in paleolimnological studies, cysts of *P. wisconsinense* have been found to be largely replaced by other cysts (e.g. *Peridinium willei*) in various lakes in Ontario, when nutrient concentrations increased (e.g. Burden et al. 1986; McCarthy et al. 2011; Krueger 2012; McCarthy and Krueger 2013; Danesh et al. 2013; and Volik 2014). Additionally, spring blooms with densities of thecae of *P. gatunense* exceeding 1,000 cells/ml (ca 300-mg chlorophyll m^{-3}) are associated with high nutrient flux in Lake Kinneret, Israel (Sukenik, 2002). Peak OC values are consistent with eutrophication associated with this lowstand, and increasingly less negative $\delta^{13}C$ values and relatively low OC/N ratios in the basin record high productivity (Figure 2.4).

In the deep basin core, the mid-Holocene lowstand is recorded by increased terrigenous flux attributed to erosion of exposed sediments along the lake margin; this is evident in measurements of dry bulk density (DBD) and OC/N indicating an increased contribution from terrestrial organic matter, likely reworked from the watershed (Figure 2.4). The establishment of a thriving, relatively high-diversity thecamoebian assemblage (SDI= 2.0-2.1) rich in *Difflugia oblonga, Centropyxis constricta "*constricta" and *Pontigulasia compressa* in the basin is evidence of organic-rich sediments and relatively high bottom water DO during the hemlock minimum (Patterson et al. 1996; Patterson and Kumar 2002).

Acoustically transparent sediments drape the entire pond above the green reflector, recording rapid deepening to modern lake levels as hemlock expanded in response to wetter conditions beginning ~3,500 years ago and peaking ~2,000 years ago (Figure 2.4). Benthic thecamoebians record the redevelopment of the anoxia evident in declining $\delta^{34}S$ in the basin core in response to deepening water and high biological oxygen demand (BOD) resulting from very high primary productivity in the water column (peak dinocyst concentrations in the basin core);

high populations of *Centropyxis aculeata, Cucurbitella tricuspis,* and the ciliate *Codonella cratera,* are similar to those found in other eutrophic, oxygen-stressed environments (e.g. Roe et al. 2010; Barbieri and Orlandi 1989). Thecamoebian populations subsequently increased dramatically in the margin core and even, to a lesser extent in the basin core (Figures 2.2, 2.3). They record a decrease in anoxia consistent with the increase in $\delta^{34}S$ (Figure 2.4), although the planktonic ciliate *C. cratera* and thecamoebian taxa able to tolerate low DO suggest eutrophic conditions and at least seasonal anoxia probably driven by BOD caused by decay of the abundant phytoplankton evident in the dinocyst record from the basin core (Figure 2.2). The lake level history of Sluice Pond corresponds to a climate-driven lowstand dated from 2,000 – 1,275 calendar years BP in both Lake Ontario (McCarthy and McAndrews, 1988) and Lake Erie (Pengelly et al.1997). This allowed us to refine the late Holocene lake level history of New England which was largely unconstrained in the reconstruction of Webb et al. (2004) as shown in Figure 2.4.

An increase in $\delta^{15}N$ in recent (ragweed-rich) sediments indicates a new source of nitrogen, likely animal waste, and a decrease in OC/N due to dilution by siliciclastic inputs (Figure 2.4) is attributed to anthropogenic impact in ragweed pollen-rich sediments deposited over the last 300 years (Hubeny et al. in press). A further intensification of bottom water anoxia is recorded by low $\delta^{34}S$ values and by the dominance of *Cucurbitella tricuspis* and *Codonella cratera*, protozoan taxa capable of withstanding anoxic, eutrophic conditions and high heavy metal concentrations in sediments due to their planktonic ecology (Medioli and Scott 1983; Scott and Medioli 1983; Patterson et al. 1985, 1996; Hunt and Chein 1983; Kling 1998). The increase in *Peridinium wisconsinense* in both the marginal and basin cores is attributed to the dilution of nutrients in response to dam-building in the 17th century and subsequent increase in lake levels (Figures 2.2, 2.3).

Conclusions

Synchronous changes in the phytoplankton, zoobenthos and zooplankton records in two cores from Sluice Pond, MA record major changes in nutrient levels and bottom water oxygenation that are consistent with interpretations based on seismic-, pollen-, stable isotopic-, and litho-stratigraphy (Hubeny et al. in press). These contributed to a better understanding of the hydroclimatic history of southern New England, identifying a probable lowstand between ~2,000

and 1,300 cal BP also seen in the lower Great Lakes region (McCarthy and McAndrews, 1988; Pengelly et al. 1997) in addition to the early and mid-Holocene lowstands, peaking around 8,500 and 4,200, respectively. The greatest limnological change was cultural eutrophication and resulting BOD-induced anoxia resulting from European colonization. The exceptionally abundant and diverse record of dinocysts spanning 11,500 years in the basin core (SP09 KC2) and over the last several centuries in the marginal core is attributed in part to low DO promoted by the basin geometry conducive to meromixis and by high BOD resulting from high phytoplankton productivity.

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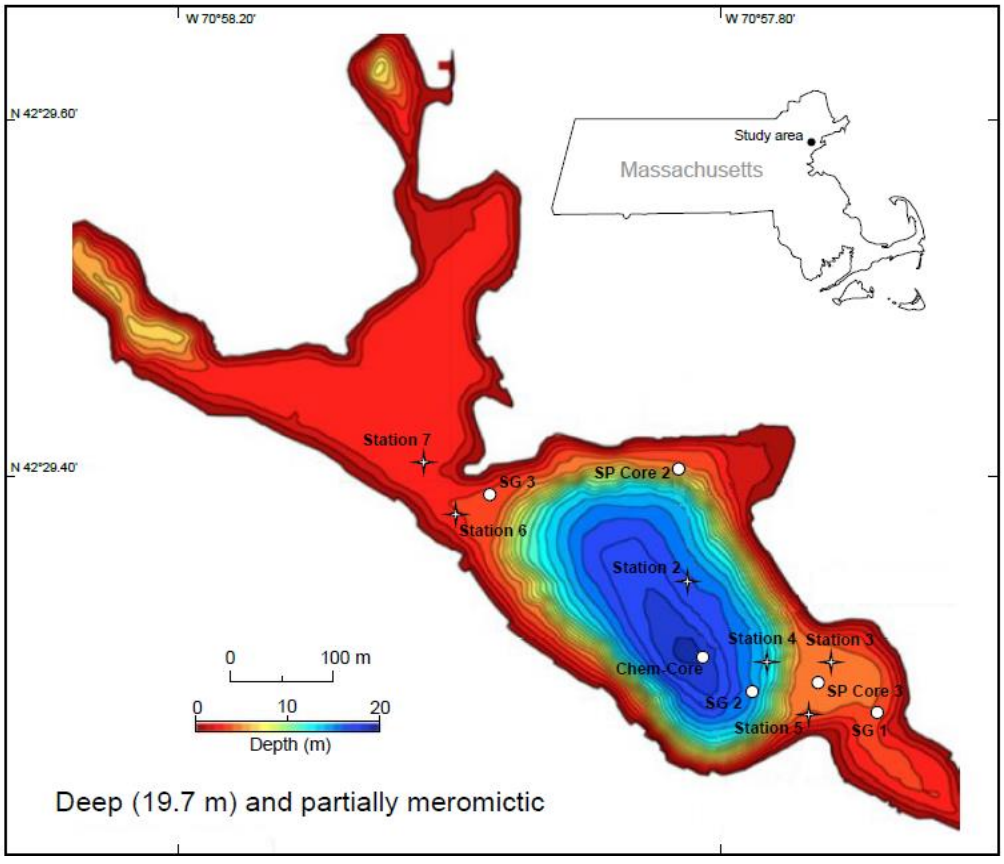
Chapter 3: Spatio-temporal distribution of algae and protozoans in anthropogenically impacted sediments: a study from Sluice Pond, MA

3.1 Introduction

It was hypothesized that the presence of protozoans and algae in a spatial and temporal study of a freshwater lake would respond to anthropogenic activity such as heavy metal pollution and increased industrialization therefore; the purpose of this study was to examine the spatial and temporal distribution of dinocyst and protozoan assemblages in anthropogenically impacted sediments. Sluice Pond is small (18 ha), deep (*Zmax* 19 m) and partially meromictic urban pond located in the city of Lynn, MA (Hubeny et al. in press) (Figure 3.1). The lake is largely recreational, where people can freely access fishing, swimming and boating.

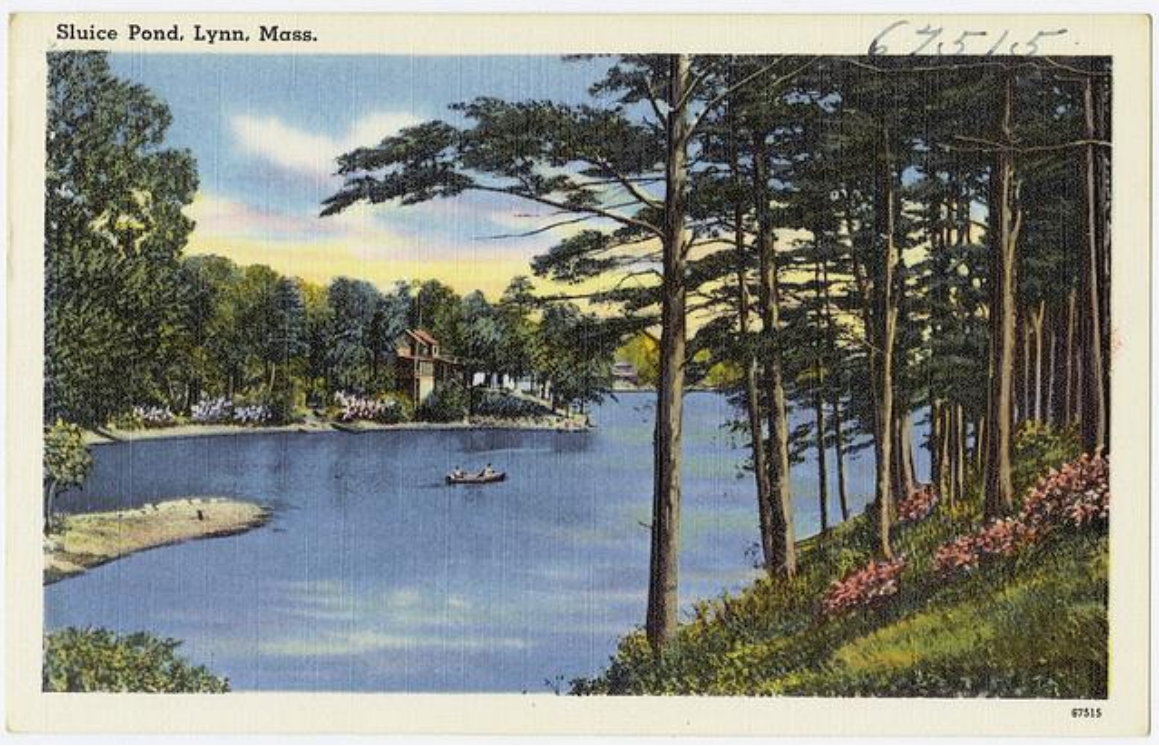


A

B 

Figure 3.1: A) A bathymetric map of Sluice Pond, MA (modified from Hubeny et al. in press) showing sampling locations. Most of Sluice Pond is relatively shallow (average depth= ~6 m) with the exception of the deep basin (20 m). SG 1 - 3 were obtained using an Ekman grab, SP Cores 2 and 3 using a Rawley-Dahl corer and Chem-Core using a gravity corer. Stations depicted with star symbols indicate water chemistry measurements and future microfossil analysis locations. B) An aerial photograph of modern day Sluice Pond surrounded by housing developments, a golf course and two major roads (Lynnfield Street to the south, and Jenness St to the north)

The municipality of Lynn was first settled in AD1629 by Puritan immigrants (Faler 1981). In AD1635, the township of Lynn began the production of shoes and tanning leather (Faler 1981). This shoe-making enterprise allowed the town to expand into a city by the 19th century. Early 20th century postcards from Lynn depict Sluice Pond as a recreational site surrounded by forest (Figure 3.2). Development around Sluice Pond likely occurred from AD1900 to AD1930 due to population expansion. At the height of Lynn's popularity, the population grew from 68,513 in AD1900 to 102,320 in AD1930 (United States Census, 2010). This substantial urban population expansion affected water quality, recorded by algal and protozoan microfossils in the lake.

A 

B 

Figure 3.2: A) Sluice Pond postcard from AD1933 (http://farm3.staticflickr.com/2371/2380472859_a2d44bf595_z.jpg?zz=1)
B) Modern day Sluice Pond

Nutrient enrichment (leading to eutrophication) is a common water-quality problem affecting freshwater systems (Schindler 2006). Although phosphorus (P) and nitrogen (N) can be introduced into the environment through natural processes, the introduction of excess nutrients into freshwater environments can cause irreversible damage and unsightly algal blooms (Schindler 1974). The cause of cultural eutrophication was greatly debated in the 1960s. Schindler (1974) and colleagues determined that the addition of phosphorus to Lake 226 in the Precambrian Shield of Ontario caused algal blooms. Additional research on Lake 304 by Schindler (1974) determined that addition of phosphorus increased chlorophyll *a* (algal productivity), while the introduction of carbon and nitrogen in subsequent years had no impact on chlorophyll *a* production. Therefore, it was concluded that phosphorus was the limiting nutrient in freshwater environments (Schindler 1974). This increased algal productivity caused dissolved oxygen (DO) to decrease as a result of biochemical oxygen demand (BOD) (Diersing 2009). In Sluice Pond's recent history, herbicide has been added to mitigate excessive algal blooms.

The impact of heavy metals on microorganisms has been well documented in freshwater systems (e.g. Reinhardt et al. 1998; Kumar and Patterson 2000; Patterson and Kumar 2002). Increased industrialization and population growth has caused an increase in contaminants such as heavy metals to enter freshwater systems that are often a source of potable drinking water for the population. Benthic organisms such as bottom-feeders and some microorganisms accumulate toxins at a faster rate than organisms living in the water-column (CPHA 1977). The mechanisms by which heavy metals affect microorganisms are not clear. Some researchers have suggested that heavy metals block enzyme systems and disturb cellular metabolites (Morgan and Lackey 1958).

Heavy metals such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) from anthropogenic activity can cause irreversible damage to freshwater ecosystems (see Appendix 13: Elemental Analysis). Although natural levels of heavy metals such as As, Cd, Co and other metals are found in freshwater systems, it is more prevalent in groundwater as rock formations are often a source of heavy metals. High levels of As are rare in rivers and lakes, and generally occur as a result of anthropogenic activity (EPA 2012). The increase in heavy metals corresponds to an increase in ragweed, associated

with anthropogenic activity. Heavy metals collapse food chains, as many organisms suffer organ damage, and hyperactivity (van Genderen 2005; USGS 2010). Areas in proximity to industrial activity are particularly prone to ecosystem collapse (Patterson et al. 1996). The Ministry of Environment (MOE) established sediment quality guidelines ranging from No Effect Level (NEL) to Severe Effect Level (SEL) (MOE 2008, Table 3.1) in an attempt to reduce heavy metal and nutrient input into lake systems. NEL indicates that chemical concentrations are low and do not effect sediment dwelling organisms whereas SEL indicates heavy contamination that is detrimental to most of sediment-dwelling organisms such as protozoans (MOE 2008).

Table 3.1: Sediment quality guidelines for metals and nutrients for the province of Ontario (MOE 2008).

	No Effect Level	Lowest Effect Level	Severe Effect Level
METALS			
Arsenic	-[b]	6	33
Cadmium	-	0.6	10
Chromium	-	26	110
Copper	-	16	110
Iron (%)	-	2	4
Lead	-	31	250
Manganese	-	460	1100
Mercury	-	0.2	2
Nickel	-	16	75
Zinc	-	120	820
NUTRIENTS			
TOC(%)[c]	-	1	10
TKN[c]	-	550	4800
TP[c]	-	600	2000

[a.] Values in µg/g dry weight unless otherwise noted (µg/g = ppm). Values less than 10 have been rounded to one significant digit. Values greater than 10 have been rounded to two significant digits except for round numbers which remain unchanged (e.g., 400).

[b.] "-" denotes insufficient data/no suitable method

[c.] TOC – Total Organic Carbon; TKN – Total Kjeldahl Nitrogen; TP – Total Phosphorus

Common thecamoebians (Class Rhizopoda von Siebold) in North American lakes belong to the Superfamily Arcellacea Ehrenberg, Family Centropyxidae Jung or Difflugiidae Stein. Most of these testate protozoans are benthic, although some have a planktonic phase (e.g., *Cucurbitella tricuspis* Medioli et al.). Their readily fossilizable agglutinated tests have been

found to be useful proxies of climate, pH, heavy metal toxicity, salinity, dissolved oxygen (DO), temperature and degree of eutrophication (McCarthy et al. 1995; Reinhardt et al, 1998, 2005; Kumar and Patterson 1999; Patterson and Kumar 2002; Roe et al., 2010; Neville et al. 2010, 2011; Patterson et al. 2012; Danesh et al. 2013). Similar to thecamoebians in morphology and appearance, ciliates are zooplankton belonging to Class Spirotrichea Small and Lynn, Order Tintinnida Kofoid and Campbell, Family Codonellidae Kent. The most common ciliate found in North American lakes is *Codonella cratera* Leidy (Hunt and Chein 1983; Danesh et al 2013; Volik 2014). It has been observed in sediment associated with increased eutrophication and muddy, anoxic bottom waters (Barbieri and Orlandi 1989; Danesh et al. 2013).

The cysts of freshwater dinoflagellates (Class Dinophycea Pascher, Order Peridiniales Haeckel, Family Peridiniaceae Ehrenberg) have been largely ignored in paleolimnological studies, in part due to their poorly understood taxonomy- resting cysts have been described for only 84 of the ~350 dinoflagellate species known to produce fossilizable cysts in freshwater sediments (Mertens et al. 2012). Although little paleolimnological research has employed freshwater dinoflagellates, Burden et al. (1985), Zippi et al. (1990, 1991) McCarthy et al. (2011), Krueger (2012), McCarthy and Krueger (2013), Danesh et al. (2013) and Volik (2014), demonstrated that cysts attributed to the genera *Peridinium* Ehrenberg and *Parvodinium* Carty record natural and anthropogenic change in lakes of eastern North America, and that they are particularly sensitive proxies of eutrophication.

3.2 Materials and methods

Three surface samples were taken using an Ekman grab along a transect from the southeast portion of the lake (SG 1: 42° 29.245'N x 070° 57.679' W) to the northern portion of the deep basin (SG 3: 42° 29.382' N x 070° 57.942' W) and two cores were taken from 4 m water depth in different parts of Sluice Pond using a Rowley-Dahl corer: a 74 cm long core (SP Core 2) was taken at 42° 29.401'N, 070° 57.830'W and a 111 cm long core (SP Core 3) was taken at 42° 29.286' N, 070° 57.729' W. Water depth was determined using standard "fish finder" SONAR and location using a Global Positioning System (GPS).

One short (28 cm long) core (Chem-Core) was taken by Prof. J.B. Hubeny and his students from Salem State University in the deepest part of the basin of Sluice Pond (~19-m,

42°29.300’ N, 070°57.815’ W) using a Kullenburg-type gravity core in order to measure heavy metal concentrations. Five sub-samples (taken from the surface, 7 cm, 13 cm, 20 cm and 25 cm) from this core were taken for elemental analysis at E3 Laboratories in Niagara-on-the-Lake, Ontario (results appear in Appendix 13) and analyzed using Hydr/Vap Gen EPA3005, EPA 3050B and APHA 4500/HACH heavy metal analysis. Water chemistry data (conductivity, temperature, pH, DO) was measured and tabulated at select stations by M. MacKinnon, B. Hubeny, S. Prader and F. McCarthy on June 26, 2014.

Sub-samples of 2.5 ml volume (measured by liquid displacement) were prepared for palynological analysis using a modified process for Quaternary sediments (Faegri and Iversen 1975) at the Earth Science department at Brock University from SP Core 2, SP Core 3 and the surface sediments. Samples from SP Cores 2 and 3 were taken at 5 cm intervals. Samples from the intervals in Chem-Core were prepared for palynological analysis where elemental analysis was performed. Samples were disaggregated using a weak solution (0.02%) of sodium hexametaphosphate and sieved using a 15 μm Nitex mesh. 10% hydrochloric acid (HCl) was used to dissolve carbonates in a warm water bath with a *Lycopodium clavatum* (10, 850 ± 200) tablet (batch # 006720) added to quantify total palynomorph concentrations (Stockmarr 1971). Samples were placed in a warm hydrofluoric acid (HF) (48%) bath for 20 - 30 minutes to dissolve silica particles and diatoms. The samples were rinsed using deionized water, centrifuged to remove excess HF and sieved to remove particles <10 μm and mounted on slides using glycerin jelly. As per the suggestion of Mertens et al. (2009), acetolysis was avoided as it selectively destroys dinoflagellate cysts and causes swelling of palynomorphs. A minimum of 50 cysts was counted per sample wherever possible (Patterson and Fishbein 1989) and identified using McCarthy et al. 2011; Mertens et al. 2012; Danesh et al. 2013; McCarthy and Krueger 2013. A minimum of 130 pollen grains were counted for the purposes of pollen zonation (using Key to Quaternary pollen and spores of the great lakes region by McAndrews et al. 1973). Raw counts, relative abundances, SDI and error of estimate calculations are shown in appendices 3 – 6. Chronological control was determined using well-dated pollen zonation for eastern North America (McAndrews 1994) adjusted for New England lakes (Newby et al. 2000; Shuman et al. 2001; Webb 1988, 2004), with the age of the ragweed rise associated with colonization of the area by Puritan settlers (AD 1629).

One to ten milliliter sub-samples were sieved using a three-tiered sieve (500-μm, 63-μm and 45-μm) for thecamoebian analysis and kept in water. The coarse, 500-μm sieve was used to retain large particulate matter such as bark or leaves. Following sieving, the 45 – 63μm and > 63μm fractions of the samples were analyzed separately in a Petri dish using a Leica MZ 125 microscope, counting at least 200 specimens where possible for statistical validity (Patterson and Fishbein 1989). Thecamoebians were identified using the key by Kumar and Dalby (1998). Raw counts, relative abundances, SDI, and error of estimate calculations are shown in appendices 7 - 12. The ciliate *Codonella cratera* was identified using SEM images from Beyens and Meisterfeld (2002).

Species diversity was calculated using the Shannon Weaver Diversity Index (SDI) (Shannon and Weaver 1949) in Microsoft Excel.

$$SDI = -\sum_{I}^{S}(F/N) \times \ln(F/N) \quad (5)$$

where: F= relative abundance and *N*= number of specimens

Data analysis software (PAST) developed by Hammer et al. (2001) was used to statistically analyze the relatedness of samples using constrained cluster analysis (CCA). Raw CCA data is available in the appendices.

3.3 Results

Twelve dinoflagellate cyst morphotypes were distinguished in Sluice Pond sediments, five of which are common in these sediments: cysts attributed to *Peridinium gatunense* Nygaard as well as cysts of *Parvodinium inconspicuum* (Lemmerman) Carty, *Peridinium volzii* Lemmermann, *Peridinium willei* Huitfeld Kaas, and *Peridinium wisconsinense* Eddy. The affinities of *P.inconspicuum, P. volzii*, *P. willei*, and *P. wisconsinense* are known through germination studies (Wall and Dale 1968; McCarthy et al. 2011; McCarthy and Krueger 2013) and the affinities of *P. willei* and *P. wisconsinense* have also been confirmed through single-cell LSU and SSU rDNA analysis using PCR (McCarthy et al. 2001; McCarthy et al. 2013), although this demonstrated that *Peridinium wisconsinense* Eddy is very different from other species of *Peridinium* Ehrenberg and is close to the species *Chimonodinium lomnickii* (Craveiro et al. 2011) It is also genetically and morphologically similar to the marine dinoflagellate *Scrippsiella* spp. (Matsuka, pers. comm). Cysts germinated by Craviero et al. (2011) from Aneboda, Sweden; North Jutland,

Denmark; Lake Helen, Greenland and Aviero, Portugal were attributed to *Chimonodinium* sp. These cysts are distinguishable by their thick (1.5 µm) wall and ellipsoidal/"peanut-shaped" cysts that range in size from 30 - 35 µm and were known as *Peridinium lomnickii* Wołoszynska which blooms during winter months and was found in ponds shortly after thaw (Craveiro et al. 2011). *Peridinium* cf. *gatunense* Nygaard are large, ornate cysts with a distinct ectophragm and were identified using photographic illustrations in Pfeister (1977) and Viner-Mozzini et al. (2003). This dinoflagellate is common in Ohio lakes (Carty 1993), although DNA studies are needed to confirm the identity of cysts. Thecae attributed to *Parvodonium* sp. were identified in SP Cores 2 and 3. Thecae were compared to SEM images from Carty (2008) but; this must be confirmed through germination studies. The affinities of rare cyst morphotypes are unknown and thus labeled C/D through L.

3.3.1 Spatial distribution of dinocysts and protozoans

3.3.1.1 Surface sediments

Surface sediments at Sluice Pond exhibit exceptional preservation of dinocysts with at least twelve morphotypes found in these sediments. *Parvodinium inconspicuum, Peridinium* cf. *gatunense, Peridinium volzii, Peridinium willei,* and *Peridinium wisconsinense* are present in most samples with *Chimonidinium* sp., *Peridinium limbatum* (Stokes) Lemmerman, and Cyst types C/D - L appearing sporadically across the lakebed. *P. willei* dominates samples from 38 % (at Chem-Core) to 70% (at SG 2) whereas *P.* cf. *gatunense* ranges from absent (at SG 2) to 18% at SG 3. Dinocyst concentrations range from ~11,878 cysts/ml at the basin (Chem-Core) to ~1,595 cysts/ml at SG 2.

Thecamoebians from all of the surface samples display high populations of *Cucurbitella tricuspis, Difflugia oblonga* and *Centropyxis aculeata. Codonella cratera* populations increase from ~5% of total protozoans at 3.35 m to 60% in the deep basin with total abundances ranging from ~160 tests/ml at SG 1 to ~68 tests/ml at SG3.

Based on MOE (2008) guidelines, surface sediments of Chem-Core exceeded the severe effect levels of As (43.8 parts ppm), Cu (169 ppm), Ni (180ppm), Pb (938 ppm), and Zn (964ppm). Cd and Cr did not exceed SEL.

3.3.2 Temporal distribution of dinocysts and protozoans

The presence of *Ambrosia* (ragweed) pollen above the woody particulate material in SP Cores 2 and 3 identifies these as having been deposited over the last few centuries, i.e. since substantial land clearing occurred in this area (Figure 3.3). The WPM contains large amounts of oak (*Quercus* sp.*)* and hemlock (*Tsuga* sp.) pollen, trees naturally common in Massachusetts forests (Appendix Figures 5.3, 5.4, 6.1) so it appears likely that extensive logging of the slopes around Sluice Pond occurred in the 17th century (Figure 3.3, 3.4) and that mature trees in the postcard represent secondary growth .

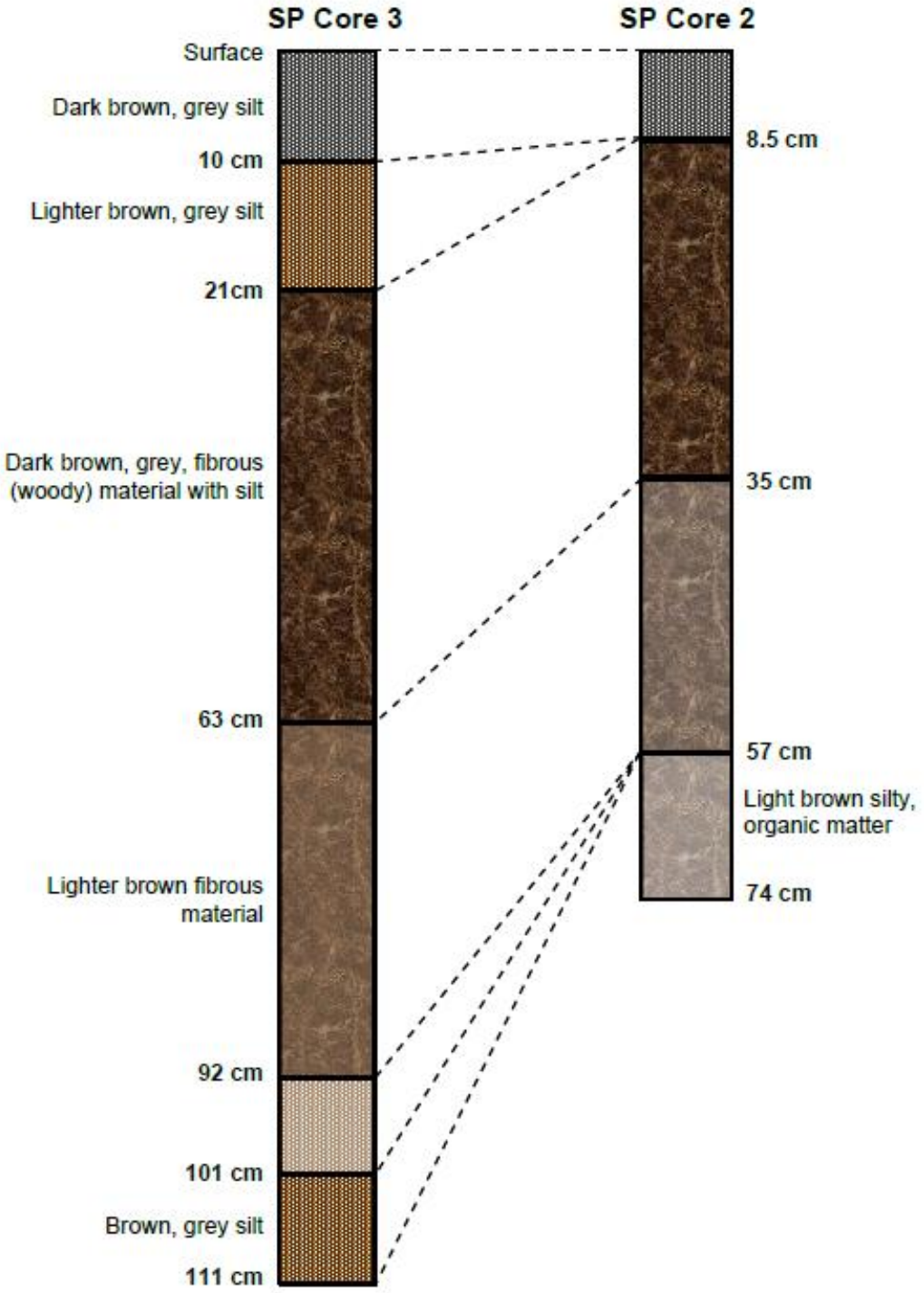


Figure 3.3: Upper portions (21 and 8.5 cm) of SP Cores 2 and 3 contain a ragweed-rich zone indicating human settlement in the area (McAndrews 1994). Below this, the presence of WPM dominates both cores until ~90 cm in SP Core 3 and ~57 cm in SP Core 2.

Figure 3.4: Dr. J.B. Hubeny holding SP Core 3 following extraction from Sluice Pond in September 2012. A clear division between the fibrous and silty material is evident near the top of the core (indicated by the arrow). The sediment-water interface was intact in both Rawley-Dahl cores.

3.3.2.1 SP Core 2

Low cyst concentrations ranging from absent to ~300 cysts/ml (in the WPM) occupy most of SP Core 2 except near the base (~2,900 cysts/ml) and near surface sediments (~1,404 - 1,951 cysts/ml). *Peridinium* cf. *gatunense* and *Peridinium wisconsinense* are sparse (statistically insignificant assemblages) in the woody particulate material. Relative abundances of these cysts range from 83% to 16%. SDI in the WPM zone ranges from 0.5 - 1.5. The presence of *Peridinium volzii* and *Peridinium willei* is noted; but their populations are sparse and inconsistent ranging from 50% abundance to absent in the WPM. At the base of the ragweed zone, total dinocyst assemblages begin to increase from ~310 cysts/ml to ~1,404 cysts/ml at the surface. *P. wisconsinense* does not recover from the base of the core and populations remain low (4-9% abundance) in the ragweed zone. *P. willei* dominates assemblages (72% relative abundance) at the surface sediments. Thecae of *Parvodinium* sp. reach a maximum of ~964 thecae/ml in the WPM and decline to 0 thecae/ml at the surface, while cysts of *Parvodinium inconspicuum* remain low ranging from 35 - 118 cysts/ml to absent in the uppermost sediment.

Centropyxis aculeata tests are abundant at the base of SP Core 2 dominating ~61% of the assemblage, decreasing upcore to sparse and statistically insignificant populations at the surface (6 tests/ml, 5.6% abundance). Thecamoebian populations remain relatively low (11 - 69 tests/ml) in the WPM of SP Core 2 ranging in SDI from 0.1 - 1.6. The ragweed zone is characterized by an increase in eutrophic protozoans such as *Cucurbitella tricuspis, Difflugia protaeiformis* and *Centropyxis constricta* Ehrenberg, *Pontigulasia compressa* Carter and *Difflugia oblonga* (SDI=1.9 - 2.0). *D.oblonga* increases markedly in this interval- from 6 tests/2.5 ml in the pre-ragweed zone to 158 tests/3.5 ml tests/ml in recent sediments of SP Core 2. A similar trend is observed in SP Core 3. The ciliate *Codonella cratera* is absent from sediments except where it appears in sparse, statistically insignificant populations at the surface sediments.

3.3.2.2 SP Core 3

Although a decrease in dinocysts is observed in the WPM zone, dinoflagllate assemblages appear less affected compared to assemblages in SP Core 2 as average cyst concentrations range from ~1,300 cysts/ml in SP Core 3 to ~690 cysts/ml in SP Core 2. Relatively high populations of *P.* cf. *gatunense, P. volzii, P. willei* and *P. wisconsinense* co-dominate assemblages from the base of the core to the base of the ragweed zone (21 cm depth) (~1800 cysts/ml at the base to ~198 -

2,600 cysts/ml in the middle of the WPM, SDI= 0.4 - 1.5) until a marked decrease in cyst concentrations is observed at the base of the ragweed zone (~133 cysts/ml, SDI=0.0). Dinoflagellate cyst concentrations in SP Core 3 range from ~133 cysts/ml to ~8,535 cysts/ml, with the highest and lowest cyst concentrations in the ragweed zone. Unlike SP Core 2, cyst concentrations of *P. wisconsinense* do not decrease near the surface of SP Core 3. Populations of *P. wisconsinense* increase from 321 cysts/ml near the base of the ragweed zone to 434 cysts/ml at the surface sediments with an abundance ranging from 4 - 50%, except at 15 cm where *P. wisconsinense* dominates 74% of the assemblage. Relative abundance of *P. willei* is 66% in the surface sediments in the ragweed zone whereas *P.* cf. *gatunense* dominates 8 - 61% of the ragweed-rich assemblage. Unlike SP Core 2 however, *P.* cf. *gatunense* does not exhibit a crash in population at the surface sediments. Cysts attributed to *P. inconspicuum* are low and reach a maximum population of ~83 cysts/ml in the WPM. Peak cyst concentrations occur when thecae concentrations are highest. A general decrease in thecae attributed to *Parvodinium* sp. is exhibited in SP Core 3 except in the pre-WPM (silty) sediment where populations are ~1200 thecae/ml.

C. aculeata tests are relatively abundant (43.8 tests/ml) at the base of SP Core 3, with an abundance of 77% (SDI=0.8), decreasing upcore to sparse and statistically insignificant populations at the surface (6 tests/ml, 1.3% abundance). Tests are sparse throughout the WPM ranging from 36 tests/5ml - 59 tests/ 2.5ml (SDI=0.7 - 1.5) until the ragweed zone which is characterized by a sharp increase in thecamoebian tests (~251 tests/ml) co-dominated by *C. constricta* ‘constricta’, *D. protaeiformis, C. tricuspis, D. oblonga* and *P. compressa* (SDI= 1.0 - 1.6). The ciliate *C. cratera* is present near the surface sediments; however, as with SP Core 2, the assemblage is very sparse (6 loricae/ 2.5ml)

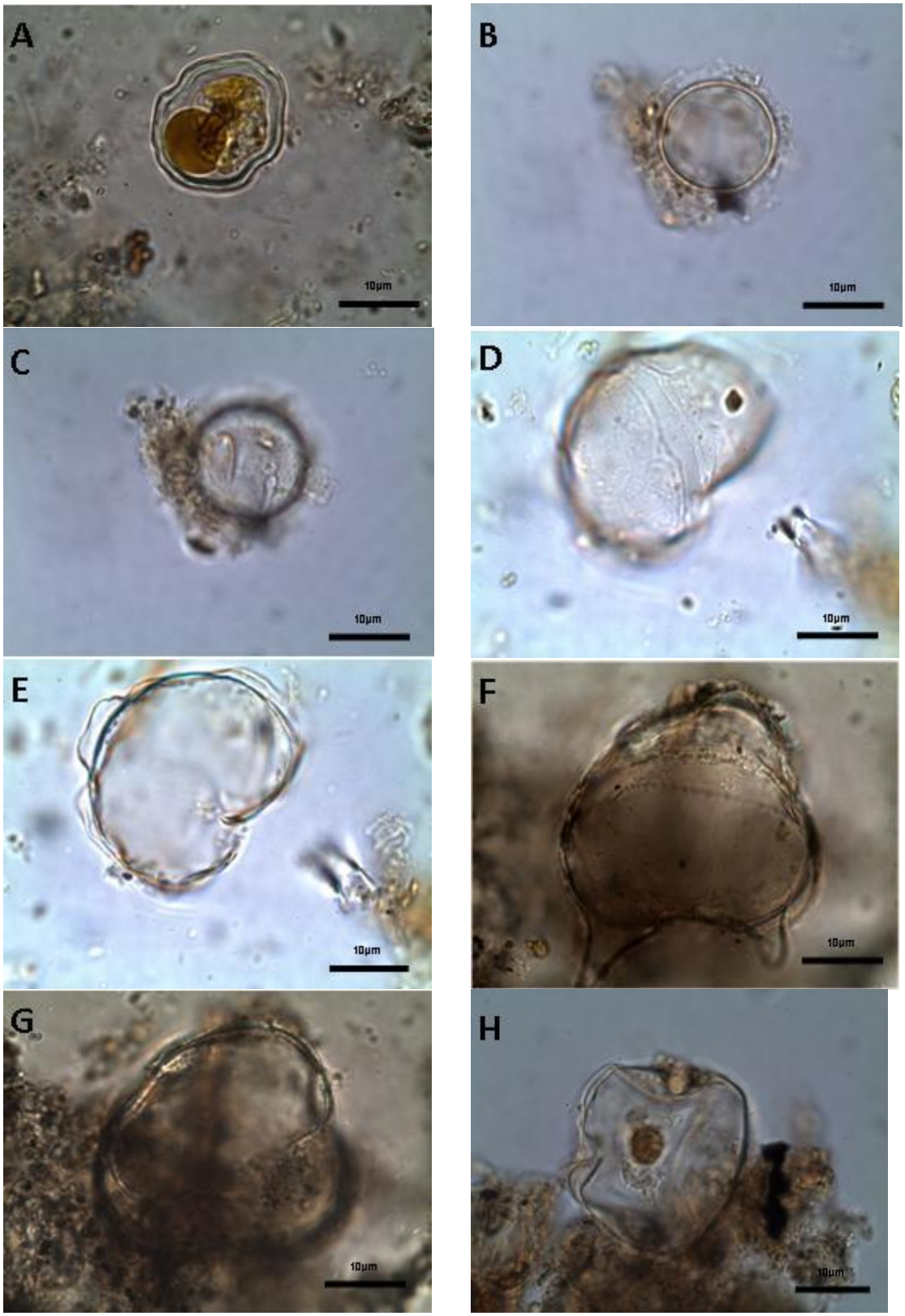
3.3.2.3 Chem-Core

The core imparted a foul, sulphur-like odour when it was extracted from the PVC tube. The 28 cm core was dark, with gytta grading upwards to the surface sediments. Chem-Core depicts a decrease in dinoflagellates at the sediment-water interface (1,828 cysts/ml), but an increase in *P. wisconsinense* in the near-surface ragweed zone (7 cm) (44,268 cysts/ml, SDI= 0.7). The five samples analyzed from the short basin core contained large numbers of the planktonic ciliate *C.*

cratera, especially where heavy metal concentrations were high (heavy metal concentrations exceeded SEL). *C. cratera* populations increased from ~21 loricae/ml at 7 cm depth to ~84 loricae/ml at the surface sediments. Total thecamoebian populations were low and decreased from ~565 tests/ml (SDI= 1.9) to ~70 tests/ml (SDI = 0.8) at the surface.

3.4 Discussion

Sluice Pond possesses an exceptional dinoflagellate cyst record from the early post-glacial to present. Compared to other freshwater dinocyst studies from southern Ontario and Minnesota, a maximum of four cyst types were identified, most attributed to *Parvodinium inconspicuum, Peridinium volzii, Peridinium willei* and *Peridinium wisconsinense* (Norris and McAndrews, 1970; Burden et al. 1986; Zippi et al. 1990, 1991; McCarthy et al. 2011; Danesh et al. 2013; McCarthy and Krueger 2013; Volik 2014). Sluice Pond contains five common morphotypes that are present in most sediments: *P.* cf. *gatunense, P. volzii, P.willei* and *P. wisconsinense, P. inconspicuum* as well as *Chimonodinium* sp., previously unidentified cyst morphotypes, named C/D, J, K, and L (Photo plate 3.1) and contains small (20 - 25 μm by 20 - 30 μm) thecae attributed to *Parvodinium* sp.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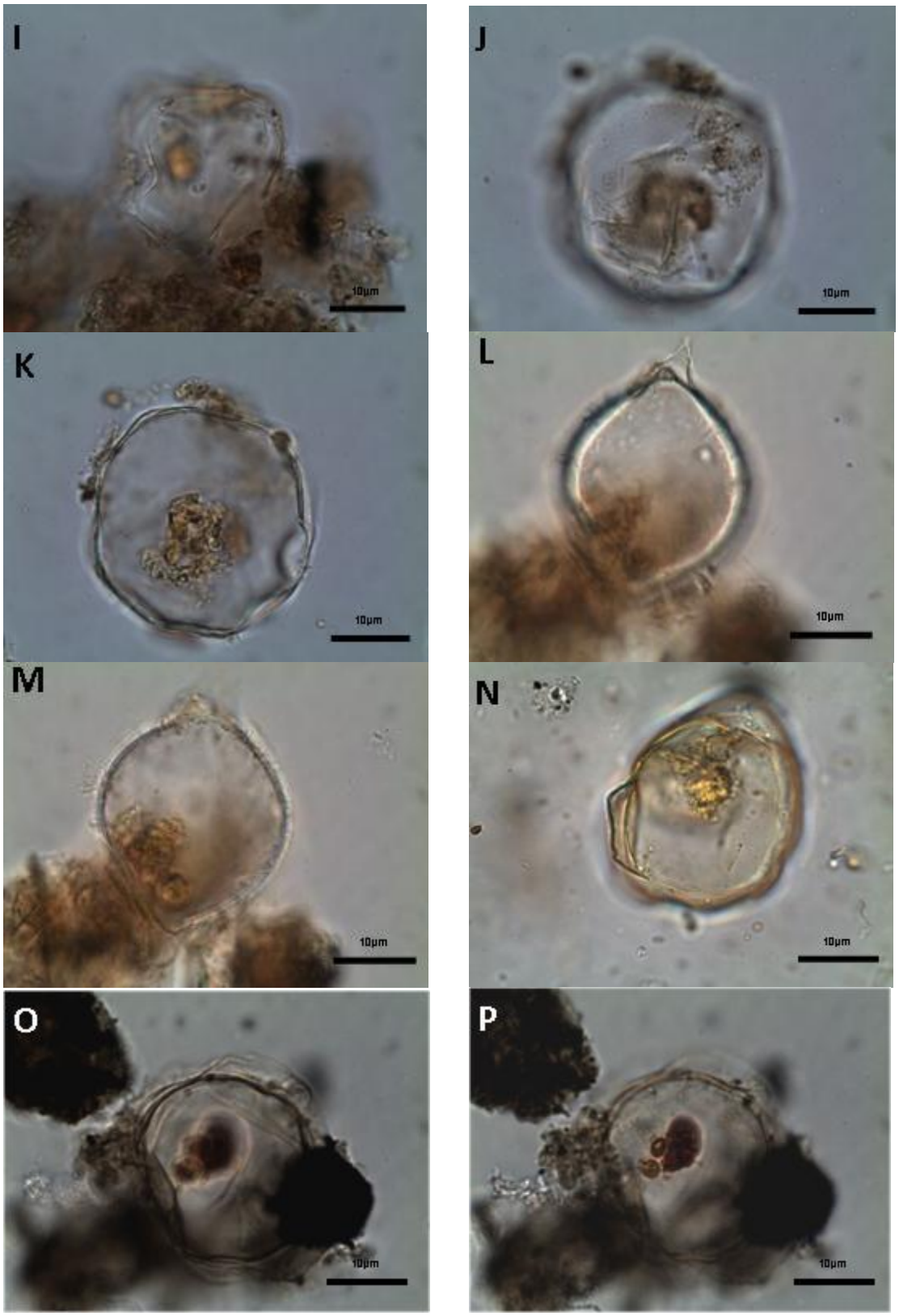
I
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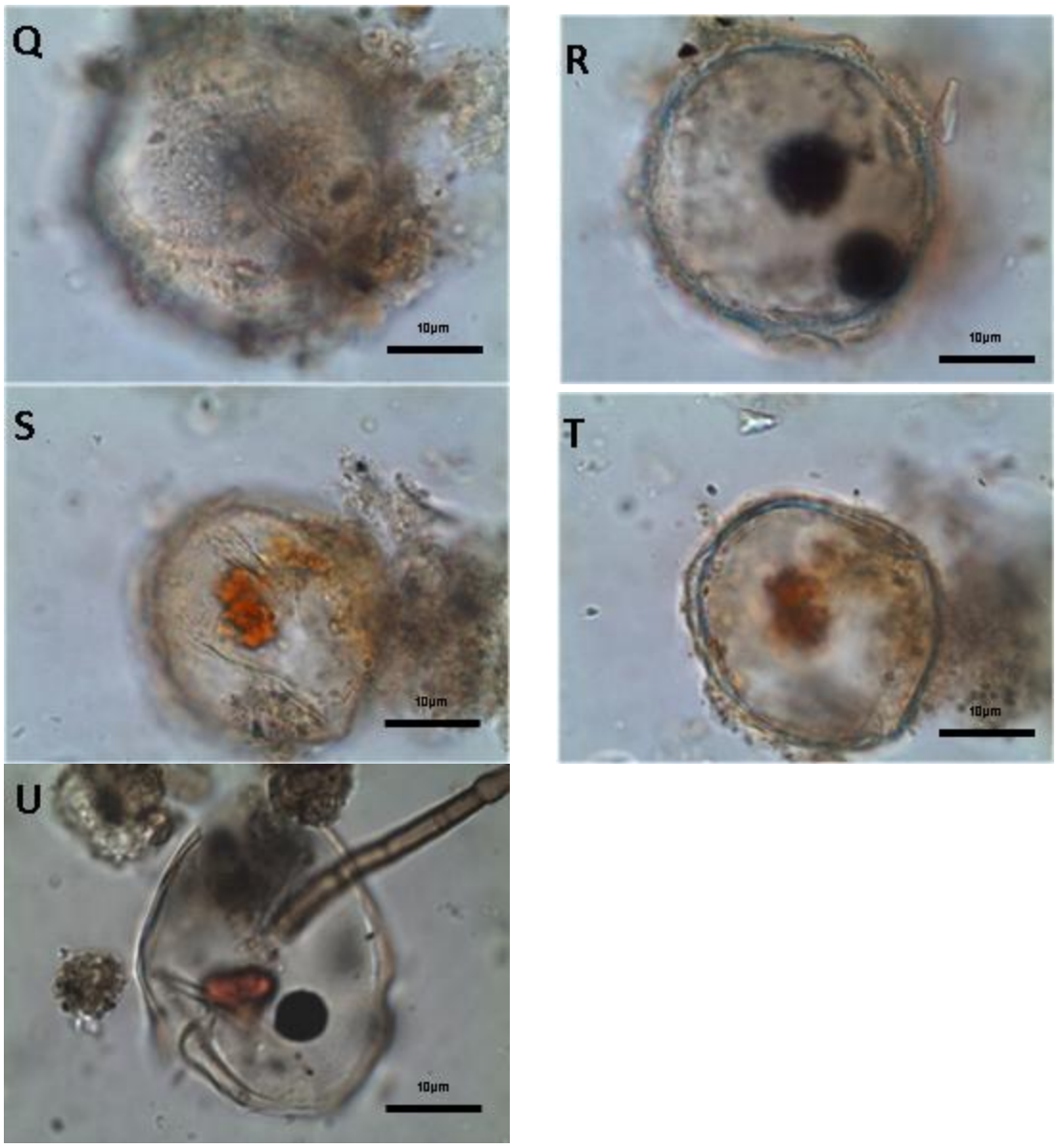


Photo plate 3.1: Exceptional preservation of dinocysts in Sluice Pond.

A) *Chimonodinium* sp. Craveiro et al. Cells are egg-shaped, small (27 - 38 µm x 22 - 32 µm) and slightly flattened dirsoventrally. The cingulum is nearly equatorial with the helmet-shaped epitheca larger than the smooth hypotheca. The sulcus does not penetrate the epitheca. Plate formula: Po, cp, x, 4', 3a, 7", 6c, 5 s, 5"', 2"" (Craviero et al. 2011). Cysts are small (longitudinal length: 20-µm), unornamented, "peanut"-shaped proximate cysts that display a visible paracingulum in dorsal and ventral view. Sample: SG 2 EF: Q17/1 B-C) *Parvodonium inconspicuum* (Lemmerman) Carty. Cells are small, and ovoid displaying a wide cingulum. The hypotheca is smaller than the epitheca. Processes are often visible on the hypotheca. Plate formula: ap, pp, cp, 4', 2a, 7'', C6, S5, 5''', 2'''' (Krueger 2012; Volik 2014). Cysts are small (12 –

20 µm) spherical, double-walled, proximate that lack ornamentation. Sample: SP Core 3 111 cm (3) EF: U43/2 D-E) *Peridinium* cf. *gatunense* Nygaard. EF: T10/4 F-G) *Peridinium limbatum* (Stokes) Lemmerman. Cysts of *P. limbatum* are described as large (92 - 104 x 64 – 82 µm), roughly pentagonal cysts with one apical and two antapical projections. The archeopyle is a lateral suture and the wall texture is granular (Mertens et al. 2012). Cysts in this study also display visible paracingulum and paratabulation. Sample: SP Core 3 10- cm (1) EF: U41/1 H-I) *Peridinium volzii* Lemmerman. Sample: SP09 KC2 S1 40 cm (1) EF: W47/0 J-K) *Peridinium willei* Huitfeld-Kaas. Sample: SP09 KC2 S1 40 cm (1) EF: X28/2 L-M) *Peridinium wisconsinense* Eddy. Sample: Chem-Core 7 cm (1) EF: H37/1

The following cysts lack formal names are described herein as cyst types. Germination is required for further classification. N) Cyst type C/D is a large (longitudinal length: 40 µm), ovoid cyst with brownish colouration, a discontinuous ectrophragm and paracingulum visible. Sample: SP Transect 7 (1) EF: U 42/2 O-P) Cyst type G is a medium sized (~30 µm), cavate (epicavate) proximate cyst, easily identified by its rounded shape, and thin, continuous ectophragm. Sample: SP Core 3 0 cm (1) EF: U25/0 Q-R) Cyst type J is a large (longitudinal length: ~40 - 45 µm), cavate, proximate cyst with a visible paracingulum, thick and continuous ectrophragm and dark-brown colouration. Sample: Chem-Core 0 cm (1) EF: N43/0 S-T) Cyst type K is smaller (longitudinal length: ~25 µm) than Cyst type J, but it displays a similar shape. Sample: Chem-Core 0-cm (1) EF: M8/0 U) Cyst type L is a medium sized (~35 µm), ovoid, cavate, proximate cyst with a clear paracingulum visible in dorsal and ventral view. Sample: SP Core 3 0 cm (4) EF: S37/3

The presence of cellulosic thecae is extremely rare in palynological samples. Studies from anoxic and deep Crawford Lake determined the cyst-theca relationship between *Parvodinium inconspicuum* and *Peridinium volzii* (McCarthy and Krueger 2013). Thecae of *P. inconspicuum* were found in Crawford Lake and cysts were later germinated (McCarthy and Krueger 2013). Previous germination studies by McCarthy et al. (2011) determined the cyst-theca relationship of *Peridinium wisconsinense* and *Peridinium willei* from Honey Harbour/Georgian Bay. Despite these germination studies, however, the presence of original thecae (without germination studies) in sediments is rare (with the exception of a few exceptional environments such as Crawford Lake) as the thecae themselves are prone to degradation and are not acid-resistant.

Elemental analysis on Chem-Core indicates that the surface sediments of Sluice Pond SEL of As, Cu, Pb, Hg, Ni and Zn (MOE 2008) (refer to Table 3.1). The total phosphorus (TP) at the surface sediments exceeds MOE guidelines by 90 ppm. The decline in abundance of dinoflagellates cysts at the top of Chem-Core suggests a decline in water quality as a result of

metal loading and/or other anthropogenic activities. The addition of herbicides in recent years may have led this marked decrease in dinoflagellates in the surface sediments. Danesh et al. (2013) attributed the appearance of *P.willei* and *P.volzii* to heavy metal loading and industrial activity in post-WWII Cook's Bay. Despite this decrease in concentration upcore, Chem-Core surface sediments exhibit the greatest concentration of dinocysts per milliliter (~11,800 cysts/ml) compared to other surface sediment samples suggesting that taphonomy and mass wasting play an important role in cyst preservation. The dilution of palynomorphs in nearshore samples (e.g. SP Core 2) is a result of sedimentation from the surrounding catchment and mass wasting associated with Sluice Pond's steep slopes. The source of sedimentation is attributed to run-off from sourrounding homes and roads. This mass wasting allows palynomorphs to accumulate in the basin while attributing to a shorter SP Core 2 (compared to SP Core 3, which is ~35 cm longer).

Dinocyst populations across the lakebed are dominated by the eutrophic indictor *Peridinium willei* which was found in Honey Harbour/Georgian Bay, Crawford Lake and Lake Simcoe during intervals of anthropogenic impact (McCarthy et al. 2011; Danesh et al. 2013; McCarthy and Krueger 2013; Volik 2014). The other eutrophic indicator, *Peridinium volzii*, which prefers slightly basic pH (Olrik 1992) and which is limited to the late-spring/summer distribution (Hansen and Flaim 2007) is low in populations across the lakebed. Molecular data from McCarthy et al. (2013) courtesy of Y. Takano suggest that *P. volzii* may be a junior synonym of *P.willei*. This is consistent with the suggestion made by Popovsky and Pfeister (1990). Hansen and Flaim (2007), however, propose that the different cell shape, smaller 1' plate and the extension of the sa-plate into the episome of *P. volzii* suggests that *P. volzii* and *P.willei* are different species.

The abundance of cysts of *P. willei* reflects this dinoflagellate's ability to thrive during intervals of harsh anthropogenic impact. Danesh et al. (2013) determined that an increase in *P. willei* in Cook's Bay was associated with post-WWII urbanization and industrialization which led to an increase in TP and heavy metals such as As, Cd, Cr, Cu, Ni, Pb, and Zn. The very good relationship with depth (R^2=- 0.88) suggests that cysts of *P.willei* preserve better than cysts attributed to other dinoflagellates (Figure 3.5). Increased mobilization of siliciclastic sediments surrounding Sluice Pond, higher rate of burial and mass wasting from the steep slopes into the

basin enhance *P.willei* cyst preservation.

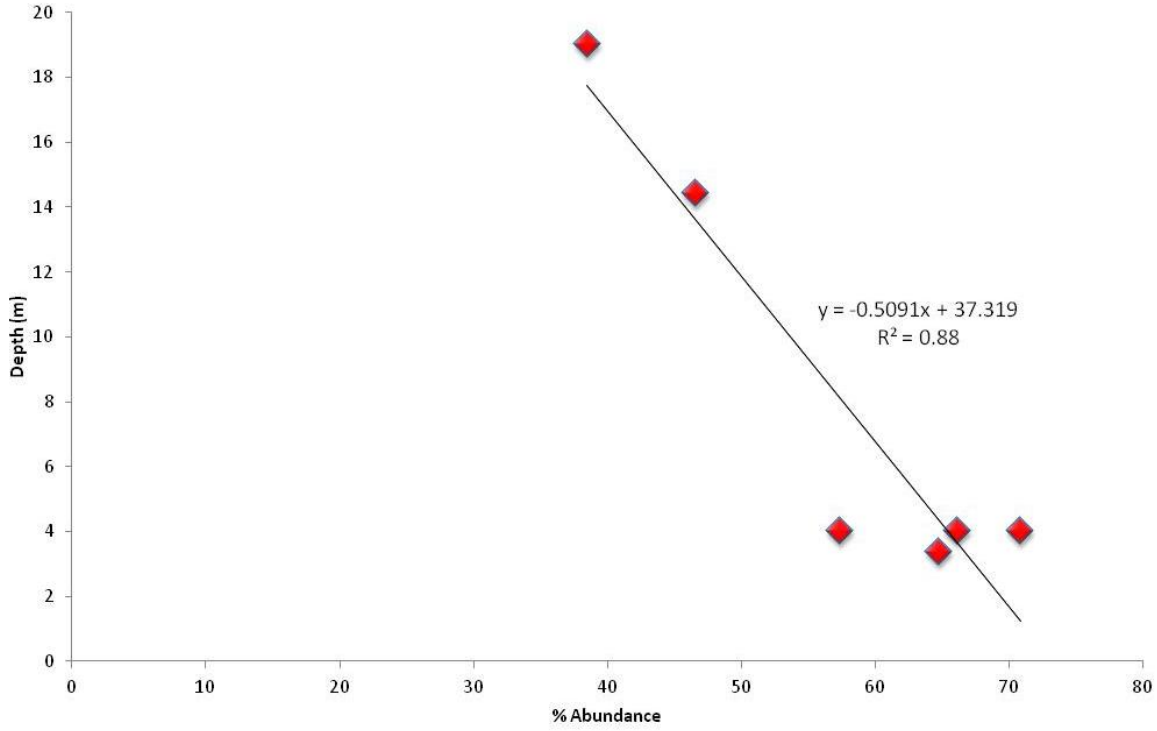


Figure 3.5: Decreasing relative abundance of *Peridinium willei* Huitfeld-Kaas reflects deep water and anoxia. Linear regression and R^2 value represent the relationship between depth below lake level and relative cyst abundance.

An inverse relationship exists between *P. willei* cyst abundance and water depth (Figure 3.5).The inverse relationship between depth and *P. willei* concentration indicates that cysts of *P.willei* preserve preferentially in shallower water compared to other cysts. Most other cysts (e.g. *Peridinium* cf. *gatunense*) have a lower probability of preservation along the shallow, oxygenated margins (Figure 3.6, additional parameters available in Appendix 15) of Sluice Pond and are less prevalent as a result of dilution from terrigenous sediments entering the pond. Although *P.* cf. *gatunense* generally reflects anoxic conditions, as its cyst is likely prone to degradation in oxygenated waters, the absence of *P.* cf. *gatunense* in the surface sediments (Figure 3.7) of the deep basin (Chem-Core) may reflect excessive heavy metal or herbicide loading. Research on Lake Kinneret suggests that selenium is the "bloom-supporting factor" in *P.* cf. *gatunense* (Zohary et al. 2012), although little research is available concerning heavy metal toxicity and *P.* cf. *gatunense.*

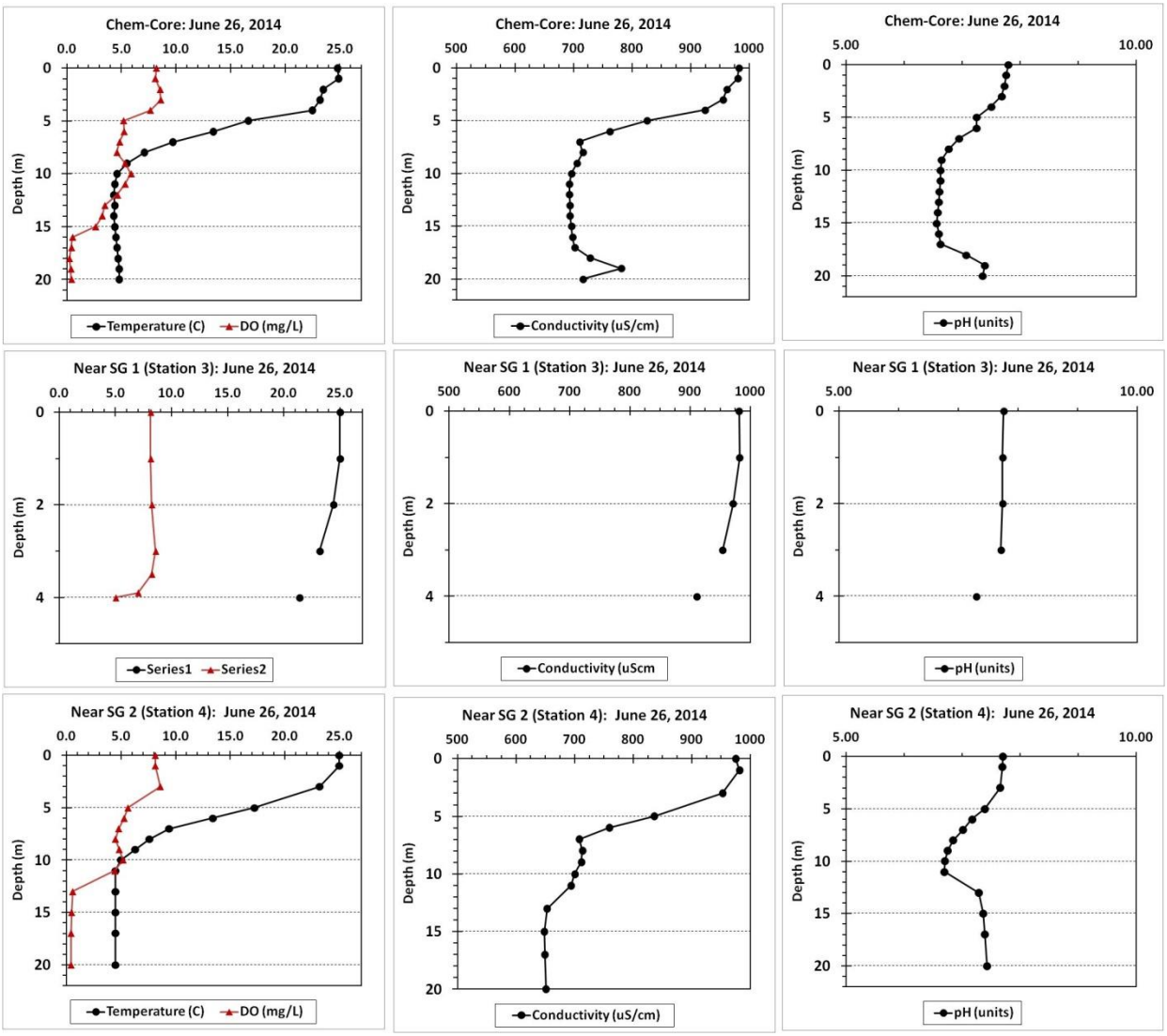


Figure 3.6: Representative charts from select locations in Sluice Pond displaying temperature (degrees Celsius), dissolved oxygen (mg/L), conductvitiy (µS/cm) and pH. The chemocline is at 15 - 16 m and the thermocline at 5 - 6 m. Low DO readings confirm anoxia in the deep basin. Data courtesy of M. MacKinnon, B. Hubeny, S. Prader and F. McCarthy.

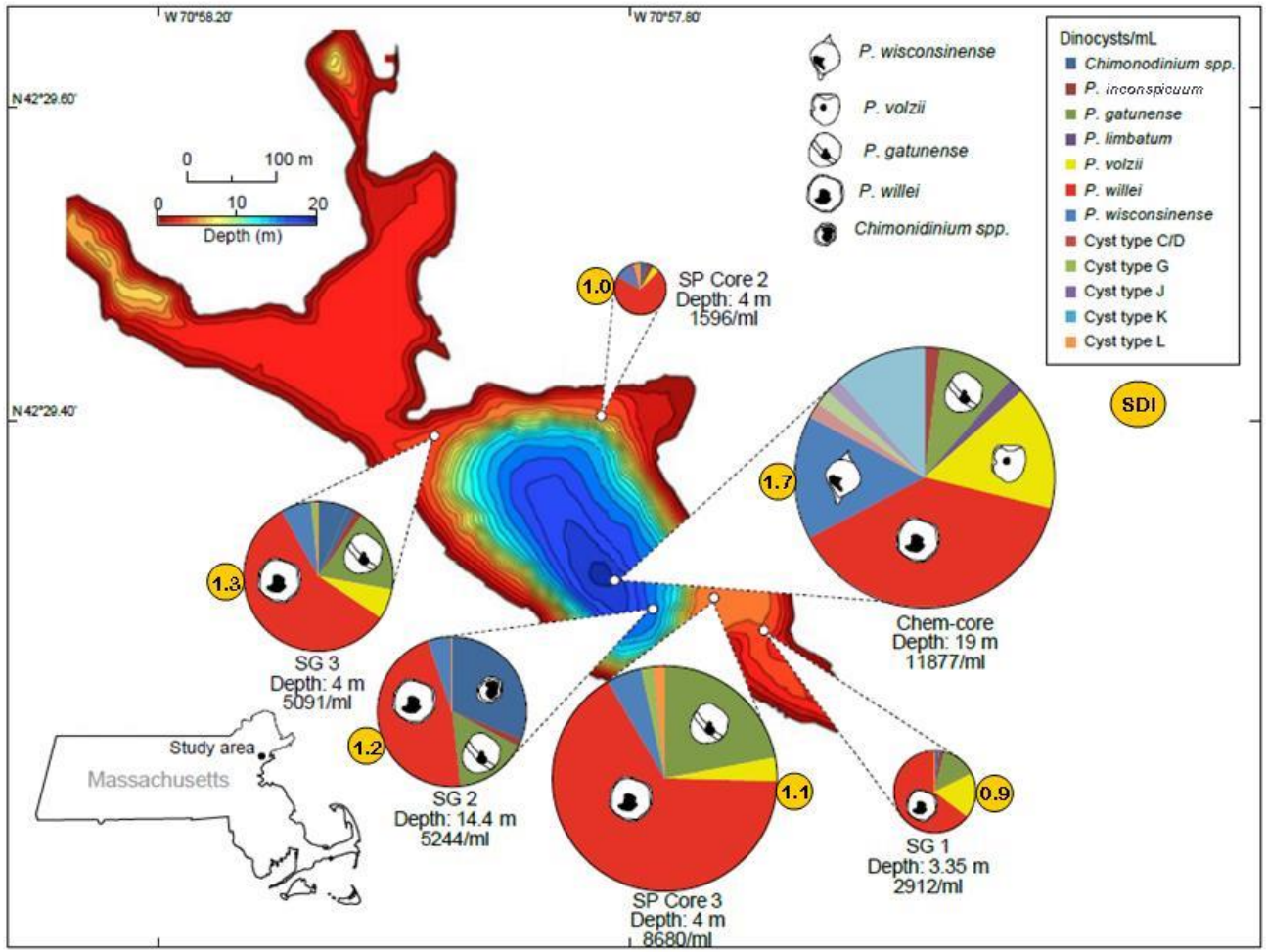


Figure 3.7: Relative and absolute abundance of dinocysts on the lakebed across Sluice Pond. The size of the pie charts is proportional to the total abundance of cysts per ml in the surface sediments. *Peridinium willei* Huitfeld-Kaas dominates the lakebed throughout, but abundance varies (likely) as a result of dilution and oxygenation. Increased mobilization of sediments from the surrounding catchment entering the pond dilute palynomorph concentration nearshore (e.g. SP Core 2 and SG1), mass wasting (as a result of the steep walls) and low DO suggest that lake bathymetry plays a role in cyst distribution.

The genus *Centropyxis* have been characterized as opportunistic, able to withstand cold temperatures, oligotrophic conditions (Schonborn 1984; Reinhardt et al. 2005), and heavy metal loading such as As and Hg (Patterson et al. 1996). As in all species thresholds, however, centropyxids cannot tolerate all heavy metal loading. For Lake Orta, in northern Italy, Asioli et al. (1996) determined that few centropyxids tolerate copper sulfate and ammonium sulfite contamination. Likewise, various strains of *Difflugia protaeiformis* were found to indicate lake acidity; however they were inhibited by As and Hg contamination. Studies completed on Peterson and Crosswise lakes in northeast Ontario (Patterson et al. 1996) determined that *Arcella vulgaris* may be a better indicator of heavily polluted substrates than centropyxids due to its affinity for heavy metals such as silver (Neville 2010). In this study, however, the presence of *A.vulgaris* at the surface sediments was virtually non-existent indicating that oxygenation of the bottom waters may play a role. Patterson and Kumar (2002) found *A. vulgaris* in the presence of well-oxygenated, silty, clay sediments, suggesting that this thecameobian prefers well-oxygenated waters. Kihlman and Kauppila (2012) studied thecamoebians as indicators of mining impact and found that *A. vulgaris* showed potential indicator value for mining contamination, although, more opportunistic thecamoebians such as *Centropyxis constricta* 'aerophila' were abundant in sites contaminated with heavy metals and sulfur (S).

Studies completed on Lake Winnipeg, Manitoba by Torigai et al. (2000) determined that *Cucurbitella tricuspis* density was associated with heavy metal loading in the Assiniboine-Red River. Torigai et al. (2000) determined that *C. tricuspis* escapes the excessive heavy metal loading by attaching itself to floating algae near the water's surface. Overall, the presence of heavy metal loading and eutrophication from the Assiniboine-Red River resulted in a decrease in thecamoebian abundance compared to the less anthropogenically disturbed northern basin. A decrease in thecamoebians at the surface sediments of Sluice Pond indicates that the threshold for heavy metal loading has been exceeded. These results are similar to studies from the Assiniboine-Red River (Torigai et al. 2000).

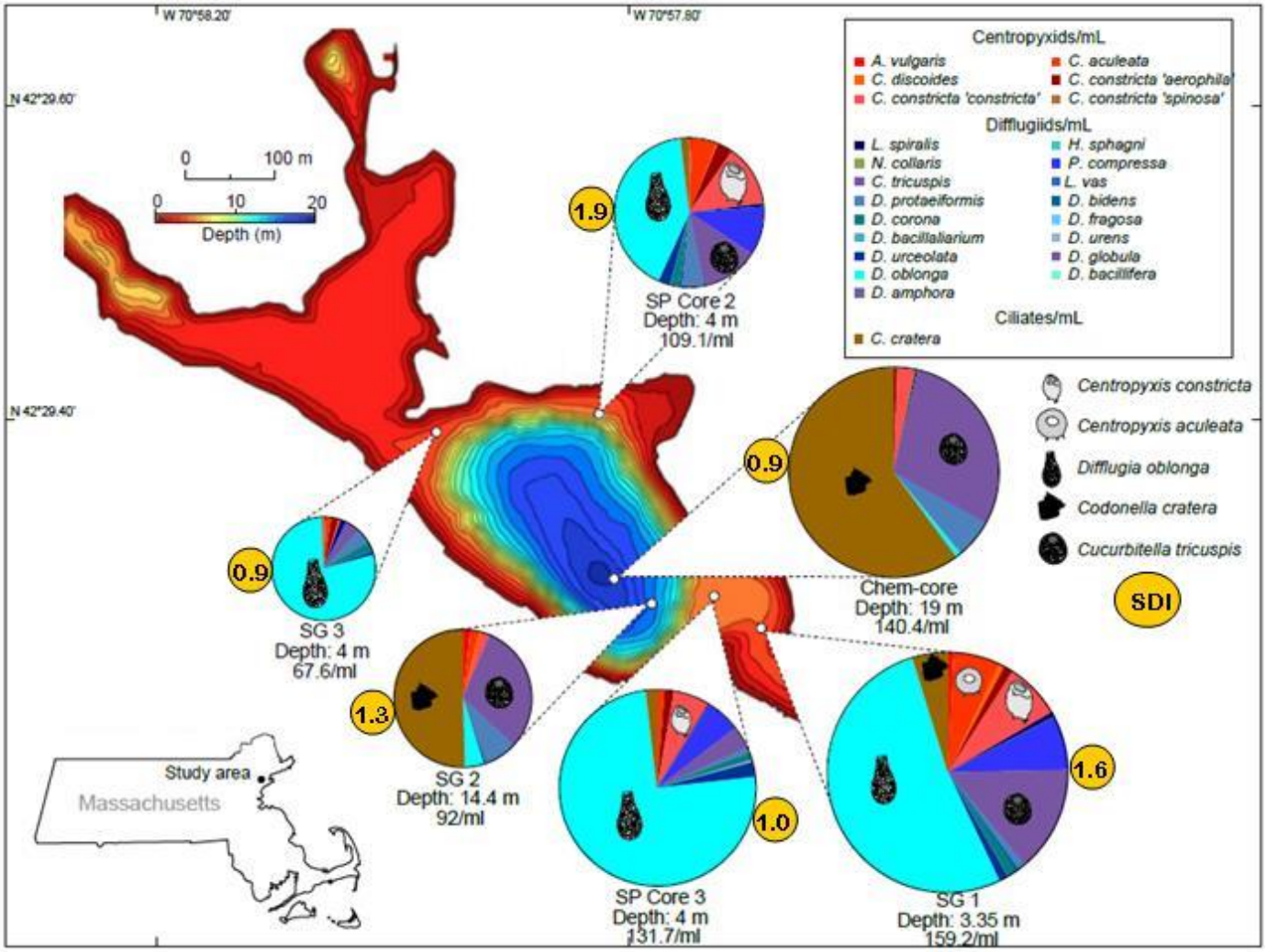
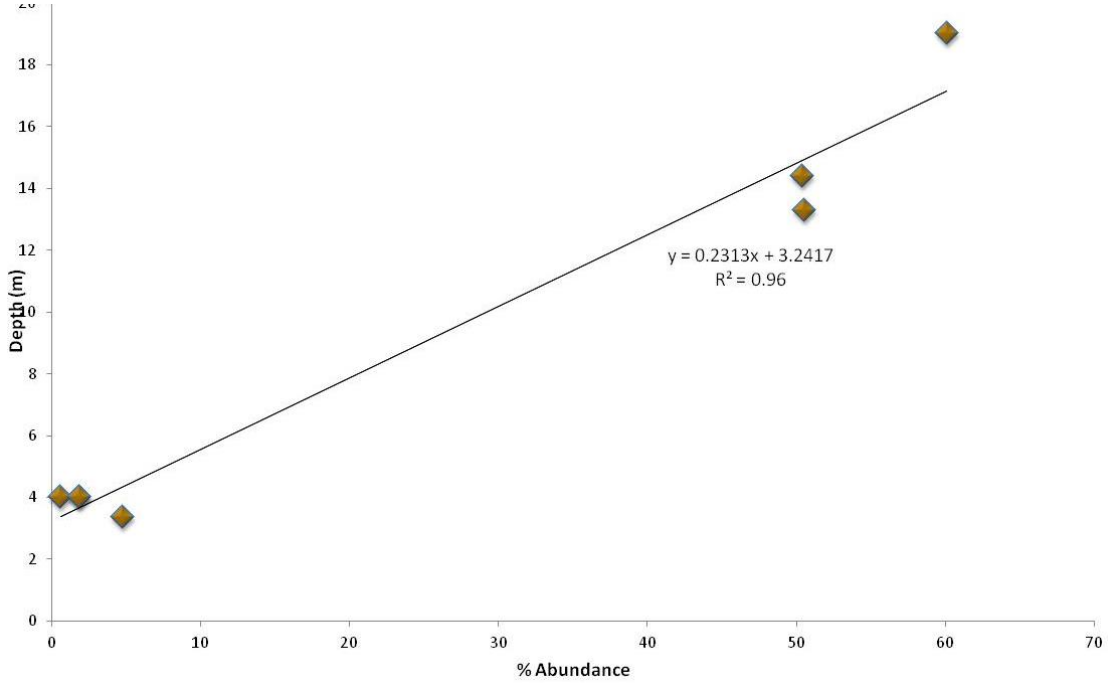


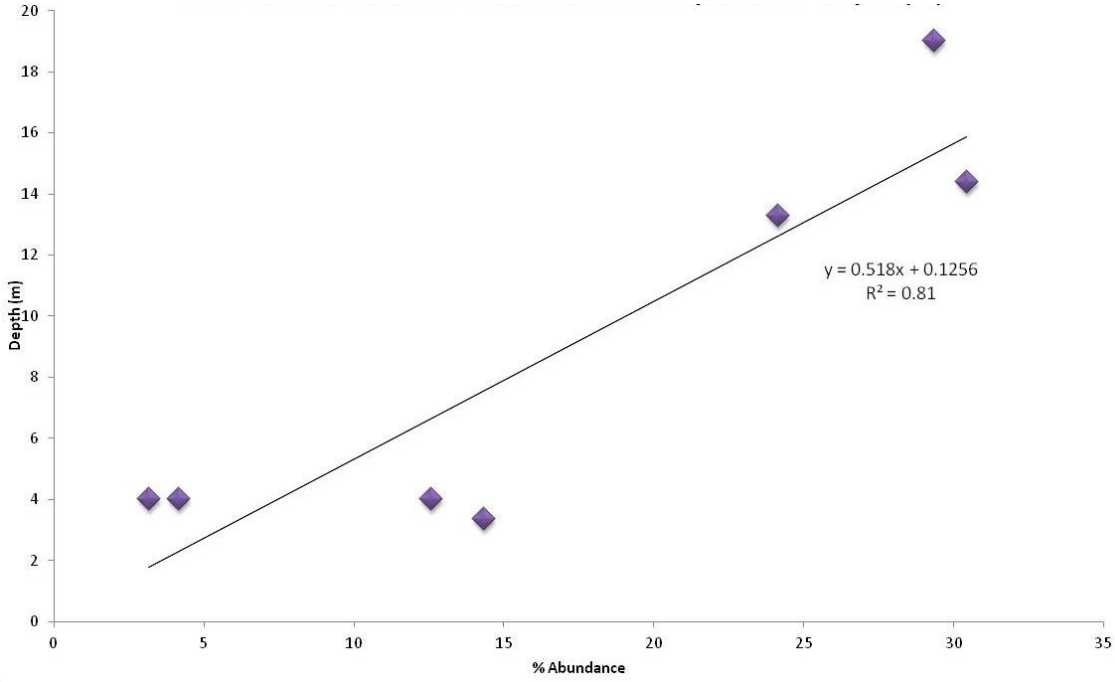
Figure 3.8: Relative and absolute abundance of protozoans on the lakebed across Sluice Pond. The size of the pie charts is proportional to the total abundance of protozoans per ml in the surface sediments. The dominance of the ciliate *Codonella cratera* Leidy in the deep basin suggests tolerance to low DO and heavy metal loading.

Sluice Pond provides an insight into the relationship between water depth and changes in protozoan communities (Figure 3.8). Studies by Barbieri and Orlandi (1989) on the Rio Grande Reservoir, Brazil indicated that *Codonella cratera* populations peaked in anoxic, muddy bottom waters, associated with pollution and anthropogenic activity. Likewise, studies by Roe et al. (2010) indicated that assemblages of *Centropyxis aculeata, Arcella vulgaris, Centropyxis constricta*, and *Difflugia protaeiformis* reflect low DO.

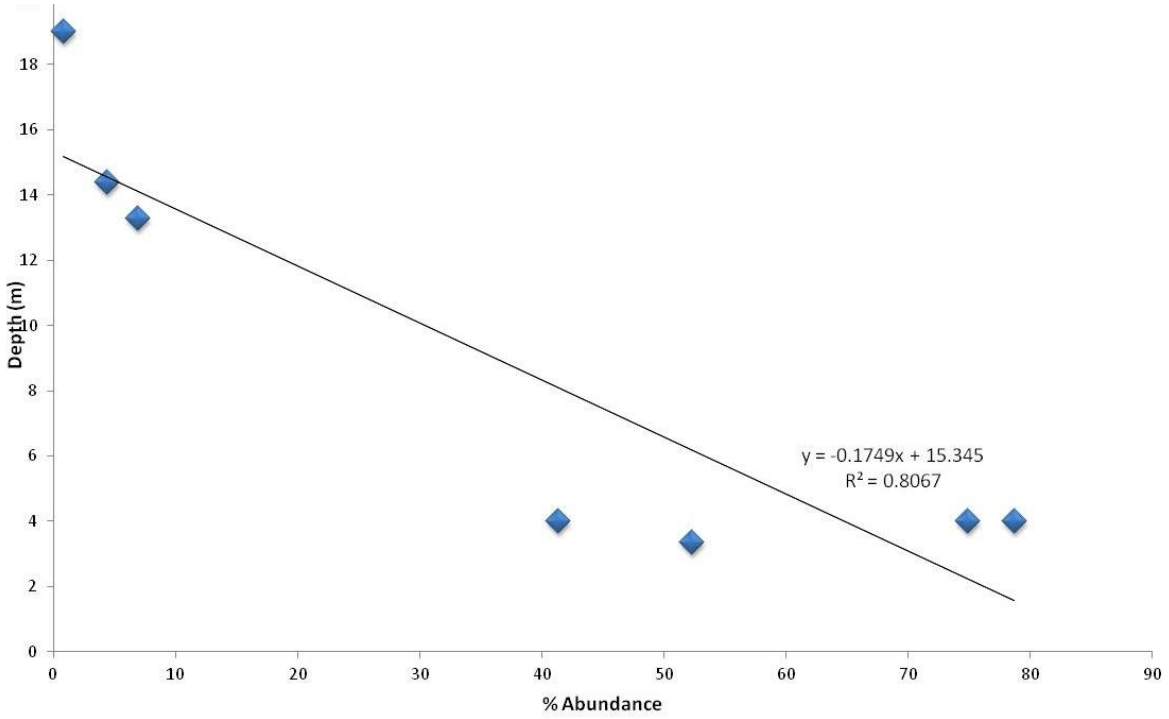
Codonella cratera and *Cucurbitella tricuspis* dominate assemblages below the chemocline whereas *D. oblonga* (R^2= -0.81) was abundant at depths less than 10 m. The exceptional relationship between depth and abundance of the ciliate *Codonella cratera* (R^2= 0.96) and thecamoebian *Cucurbitella tricuspis* (R^2= 0.81) indicates the ability of these protozoans to thrive in anoxic, eutrophic, heavy metal impacted sediments (Figure 3.9). The planktonic nature of *C. cratera* and *C. tricuspis* allows for escape during intervals of high eutrophication, or heavy metal loading (Torigai et al. 2000). *C. cratera* in Cook's Bay, Ontario was found to be present in the upper 40 cm of the ~100 cm core, coinciding with an increase in *C. tricuspis* and *D. protaeiformis* indicating progressively eutrophic conditions due to industrialization (Danesh et al. 2013). The presence of *D. oblonga* in shallower regions of the pond reflects oxygenation of the bottom waters. Volik (2014) describes the presence of *D.oblonga* in nutrient-enriched, warm environments whereas the onset of anthropogenic activity drives the arrival of *C. tricuspis* as heavy nutrient loading leads to lower DO. This shift from *D. oblonga* to *C. tricuspis* indicates that most thecamoebians (and other organisms) thrive as a function of oxygen availability.


y = 0.2313x + 3.2417
R² = 0.96
Depth (m)
% Abundance
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50
60
70

A


y = 0.518x + 0.1256
R² = 0.81
Depth (m)
% Abundance
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
20
25
30
35

B



C

Figure 3.9: Percent relative abundance of A) *Codonella cratera* Leidy B) *Cucurbitella tricuspis* (Carter) Medioli et al. and C) *Difflugia oblonga* Ehrenberg versus depth (m).

Dark grey thecae attributed to *Parvodinium* sp. (Photo plate 3.2 A-B) are prevalent (average= ~222 thecae/ml in SP Core 2 and ~318 thecae/ml in SP Core 3) throughout SP Cores 2 and 3, particularly at the base of the WPM of SP Core 3 and in the WPM of SP Core 2 (Figure 3.10).These cores are from anthropogenic activity as the bottom silt-dominated portions depict an undisturbed, forested upland surrounding Sluice Pond and the WPM above the silt indicates mass clear-cutting from the early 17th century. This mass clear-cutting and subsequent rapid burial probably led to preservation of thecae and cysts.

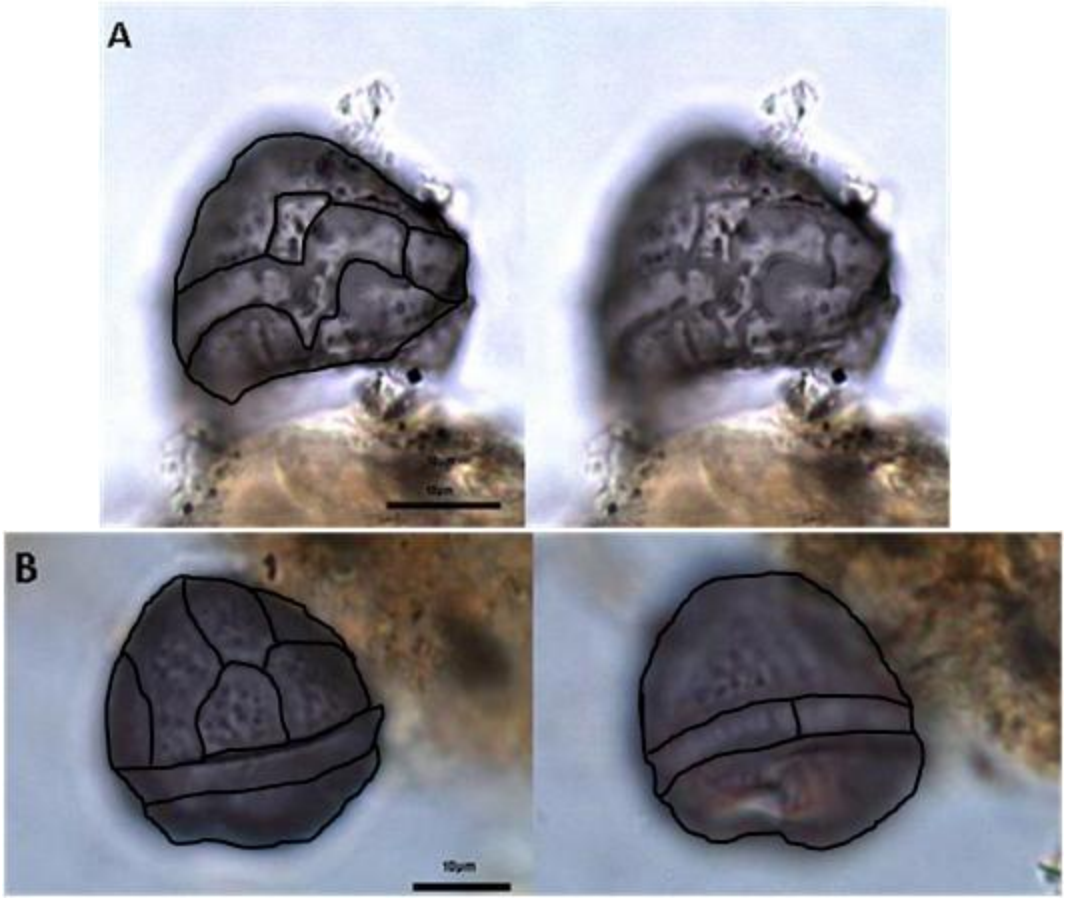


Photo plate 3.2: A-B) Thecae of *Parvodinium* sp. found in SP Cores 2 and 3 displaying dark grey colouration and outlined tabulation (A: Sample: SP Core 3 20 cm (1) EF: L31/2)B) SP Core 3 111 cm (1) EF: N29/0. These thecae are 25 by 30 µm with granulate walls and the episome larger than the hyposome. The sulcus is descending and aligned.

Preservation of rare and unknown dinocysts is attributed to rapid burial as a result of anthropogenic impact. A similar anthropogenic-induced anoxia is present in Crawford Lake where Euro-Canadian settlers increased nutrient input and caused algal blooms, leading to lower dissolved oxygen (DO) (Krueger 2012; McCarthy and Krueger 2013). In particular, the presence of the distinct *Peridinium* cf. *gatunense* has been attributed to anoxic bottom waters throughout Sluice Pond's history. This cyst was not identified in studies from Honey Harbour/Georgian Bay, Cook's Bay/Lake Simcoe (McCarthy et al. 2011; Danesh et al. 2013; Volik 2014), perhaps reflecting taphonomic or ecological differences. The presence of other unknown cysts is inconsistent; however their presence reflects Sluice Pond's exceptional preservation potential. Lower dinocyst abundance in fibrous material is due to rapid sedimentation following clear-cutting.

Dinoflagellates appear to decrease in population as the clear-cutting process continued around the catchment. Populations of *Peridinium wisconsinsnese* and *P.* cf. *gatunense* decrease in the WPM indicating that conditions are unfavourable at this time (SDI=0.5 - 1.5 in SP Core 2; 0.0 - 1.5 in SP Core 3). The proximity to shoreline and thus, close proximity to waste effluent of SP Core 2 impacted microfossil populations more than in SP Core 3 as indicated by an overall decline in *P.* cf. *gatunense*. An increase in nutrient levels is recorded by the decline in *P. wisconsinense* and increase in *P.* cf. *gatunense* and *P. willei* at the surface of SP Core 3. Although clear-cutting affected benthic protozoan and dinocyst populations from flourishing, it also promoted rapid burial, contributing to preservation. In comparison to the 74 – 111 cm anthropogenically impacted sediments in SP Cores 2 and 3, dinoflagellate assemblages from piston cores SP07 PC4 and SP09 KC 2 (Chapter 2) depict only the upper 20 – 30 cm of core were anthropogenically impacted. These sediments are dominated by silt or gytta, and lack an interval of WPM, indicating a hiatus at the sediment-water interface due to piston coring. These cores are rich in *P. wisconsinense* at the near-surface sediments, however, similar to SP Cores 2, total dinoflagellates decrease at the surface.

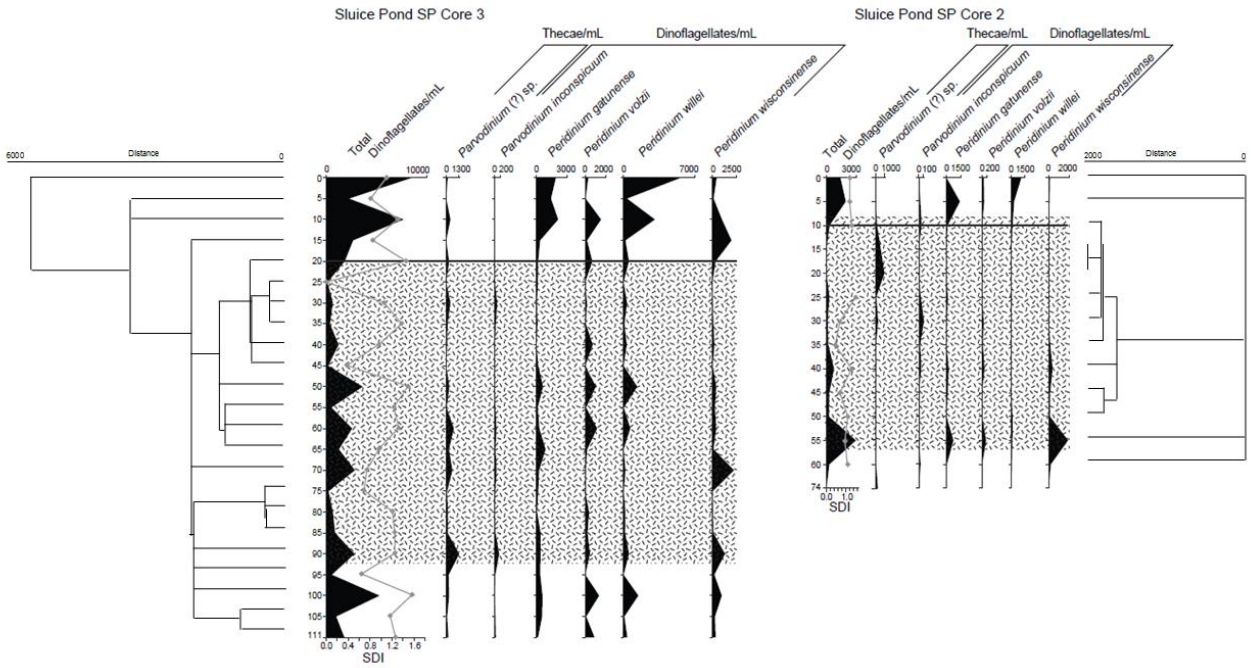


Figure 3.10: Dinocyst concentration in SP Core 2-3 against depth with CCA.Note the increase in total dinocysts near the transition between woody tissue (textured fill) and silty sediment. The fibrous pattern denotes the woody particulate material (WPM) and the solid black line denotes an

increase in ragweed. The SDI is plotted with total dinocysts per ml. SDI between 10 - 25 cm is not available for SP Core 2 due to the absence of cysts.

Thecamoebian populations record harsh conditions during the interval of mass clear-cutting (SDI= 0.1 - 1.6 in SP Core 2 and 0.7 - 1.5 in SP Core 3). Hostile conditions during clear-cutting reflect rapid burial and the development of anoxia. Other thecamoebians such as *Centropyxis constricta* 'constricta' and *Centropyxis aculeata* increase in this zone, indicating hostile, unfavourable conditions (Patterson and Kumar 2002) (Figures 3.11, 3.12). Overall, however, thecamoebian populations are low in this woody interval as particulate matter falling through the water column lowered DO.

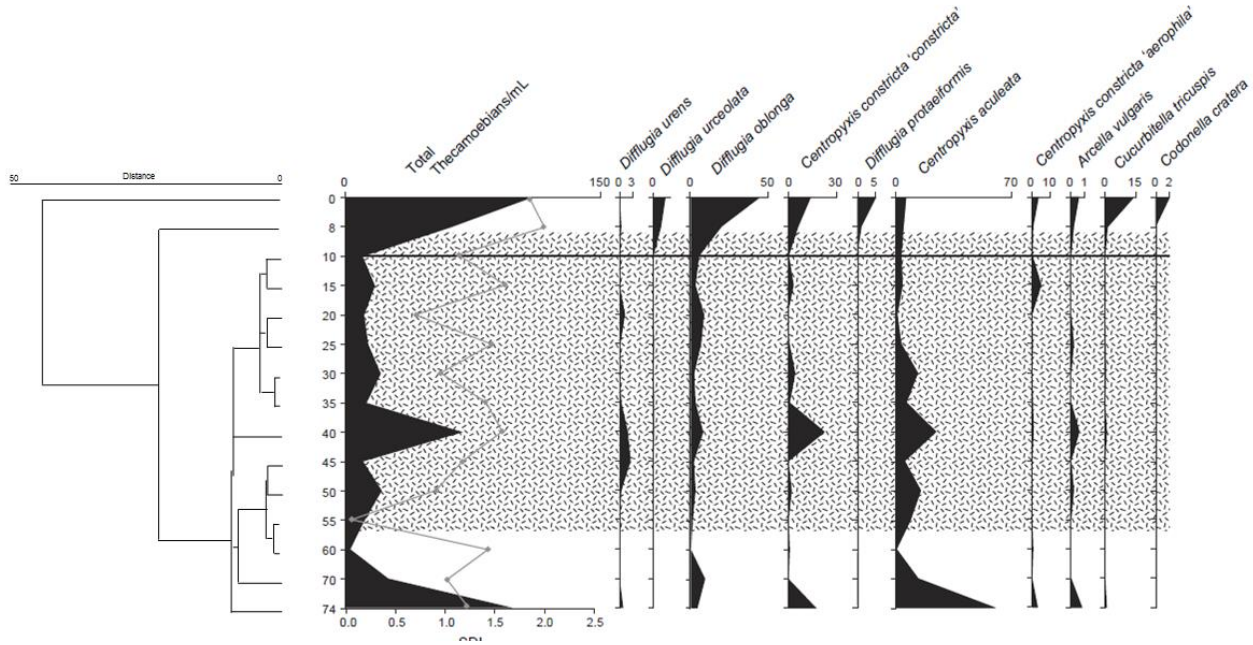


Figure 3.11: Concentration of protozoans in SP Core 2 taken at 4-m water depth with plotted CCA. Note the change from WPM (textured fill) to silty material corresponds with an increase in total thecamoebians. The fibrous pattern denotes the woody particulate material (WPM) and the solid black line denotes an increase in ragweed. The SDI is plotted with total thecamoebians per ml.

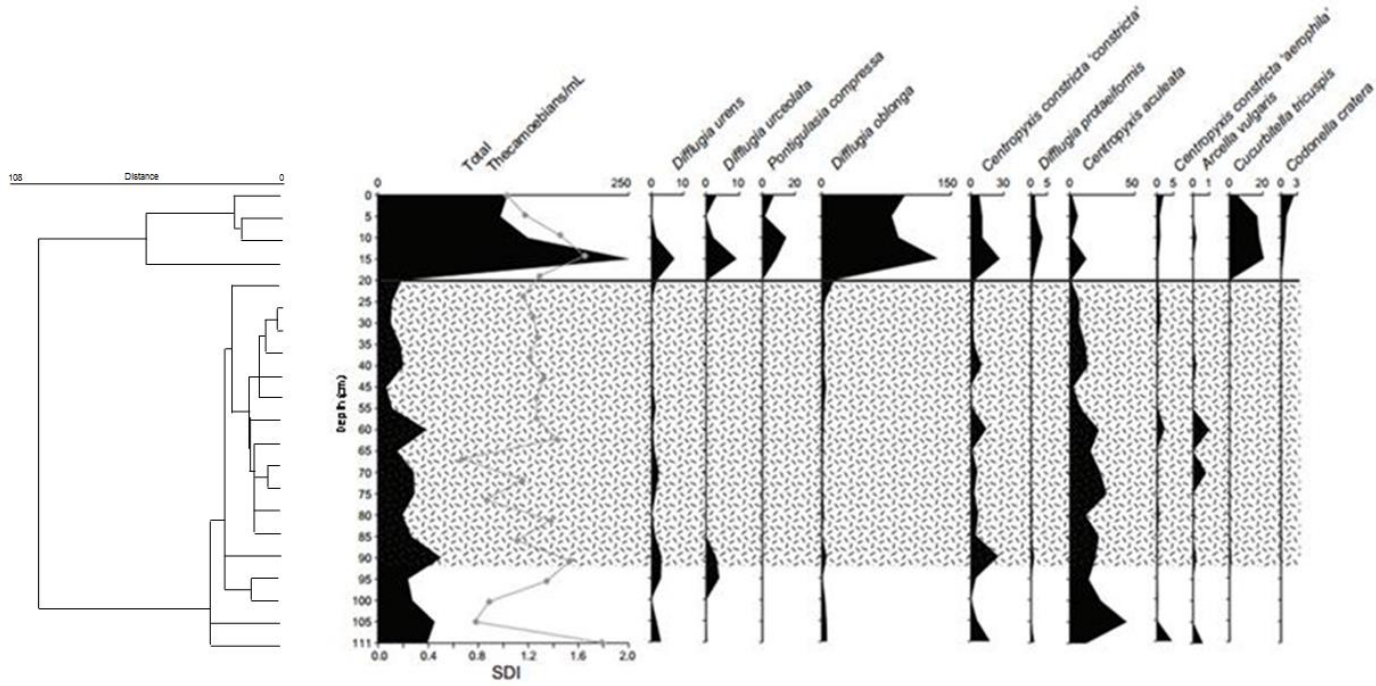


Figure 3.12: Concentration of protozoans in SP Core 3 taken at 4 m water depth with plotted CCA. A transition from less woody to more silty sediment complies with an increase in total thecamoebian assemblages. The fibrous pattern denotes the woody particulate material (WPM) and the solid black line denotes an increase in ragweed.

At the base of the ragweed-rich zone, *Difflugia oblonga* increases markedly, indicating that mass clear-cutting terminated and oxygenation of the bottom waters resumed. This corresponds to a marked increase in magnetic susceptibility (Chapter 2, Figure 2.2) at the base of the ragweed zone in the deep basin core- SP09 KC2. A slight increase in *D. oblonga* at the base of the ragweed zone in cores SP07 PC4 and SP09 KC2 indicates low DO. Ragweed-rich sediments from piston cores SP07 PC4 and SP09 KC2 indicate an increase in the ciliate *Codonella cratera* and a crash in thecamoebians, particularly *Cucurbitella tricuspis* at the surface sediments of both cores. This is similar to analysis in SP Core 3, however SP Core 2 depicts an increase of thecamoebians at the surface sediments, particularly *D. oblonga* and *C. tricuspis.*

Heavy metal analysis on Chem-Core indicates that below the surface, heavy metal concentrations are at LEL (Figure 3.13). Chem-Core is dominated by silty sediments with *Ambrosia* and other non-arboreal pollen (NAP) increasing from 13 cm to the surface. The base of this short core is occupied by *Peridinium* cf. *gatunense*, *Peridinium willei* and *Peridinium volzii*. Upcore, dinocyst populations collapse except for a peak in *P. wisconsinense* near the surface sediments attributed 17th century dam building, which led to a subsequent rise in lake levels, and dilution of nutrients (Figure 3.13). This collapse at the surface sediments may be attributed to the recent addition of herbicides in Sluice Pond in order to mitigate unslightly algal blooms.

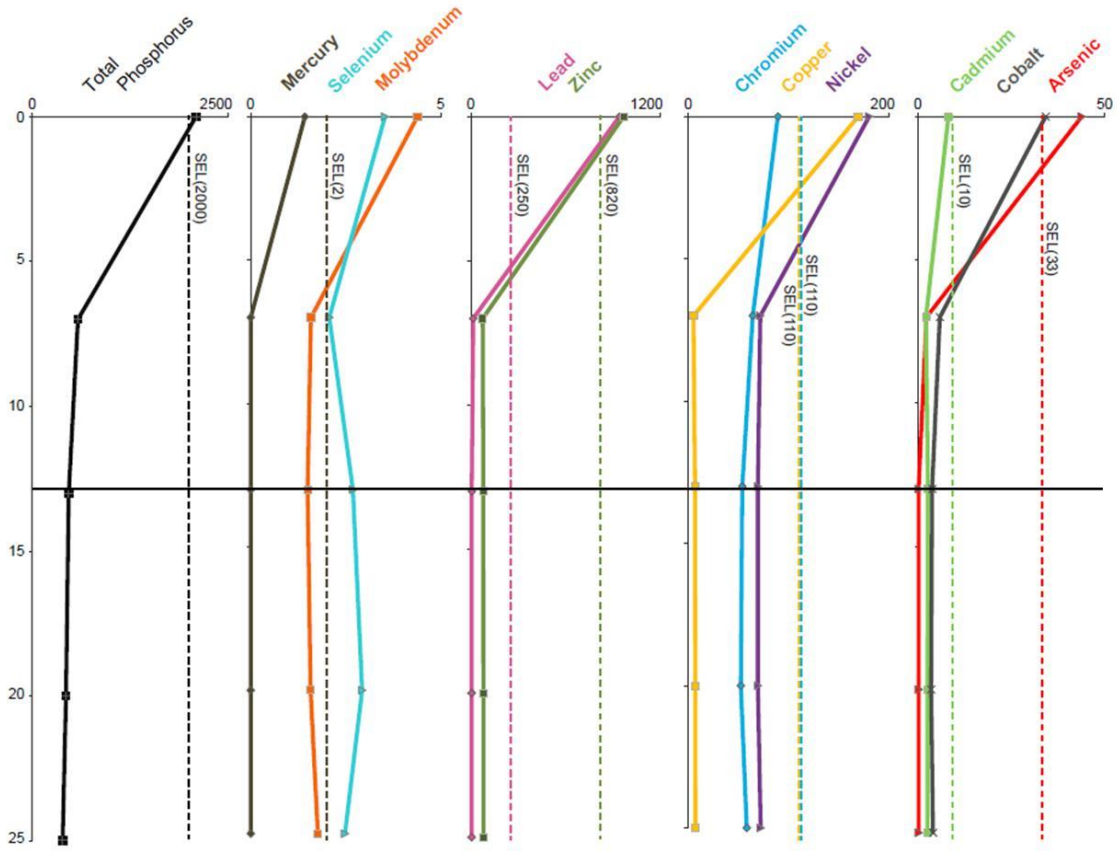


Figure 3.13: Heavy metal and total phosphorus (TP) concentrations in Chem-Core. Stippled lines denote the severe effect level (SEL) for available heavy metals and TP (MOE 2008). The black line at 13 cm represents the rise in *Ambrosia* and other non-arboreal pollen (NAP).

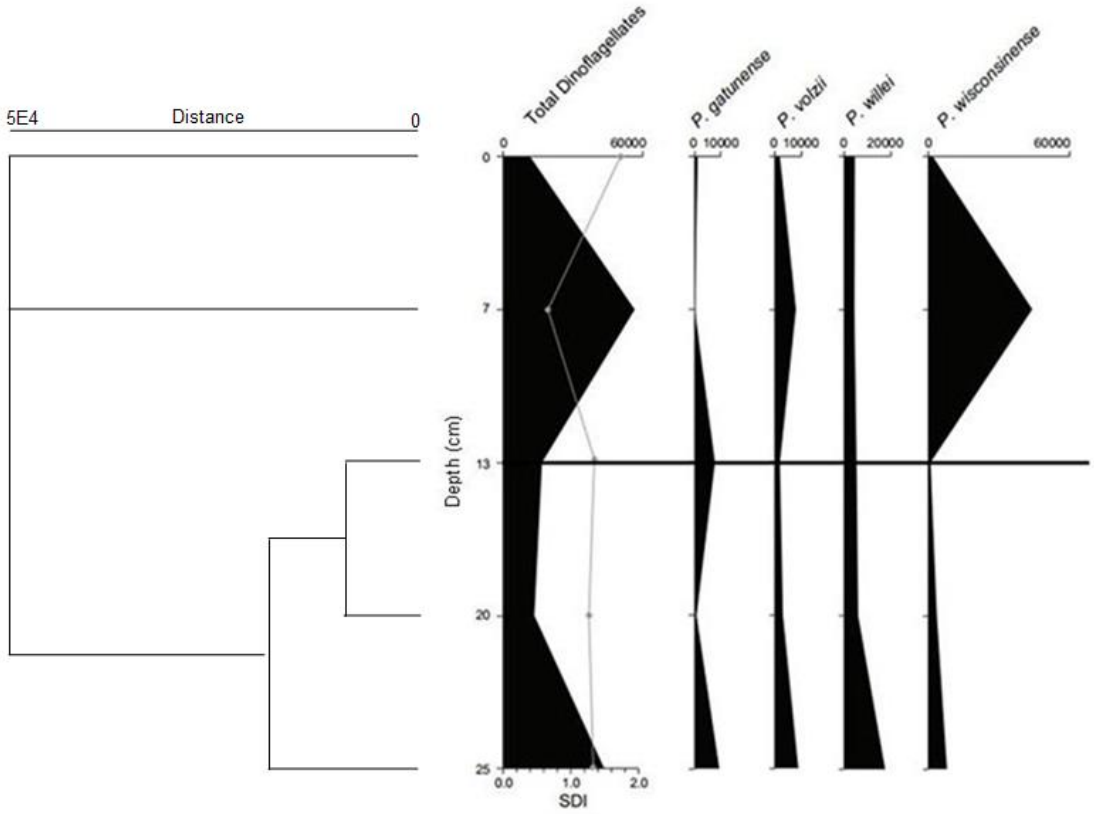


Figure 3.14: Dinoflagellate cysts per ml in Chem-Core with CCA. Note the decrease in total dinocysts at the surface sediments. The black line at 13 cm represents the rise in *Ambrosia* and other non-arboreal pollen (NAP). SDI is plotted with total dinocysts per ml.

As with the dinocyst populations, thecamoebian populations decrease upcore, particularly the eutrophic indicator *Cucurbitella tricuspis*. At the surface sediments, the ciliate *Codonella cratera*, dominates (Figure 3.15). The increase in *C. cratera* at the expense of *C. tricuspis* at the top of Chem-Core indicates that only *C. cratera* can tolerate heavy metal toxicity in modern-day Sluice Pond.

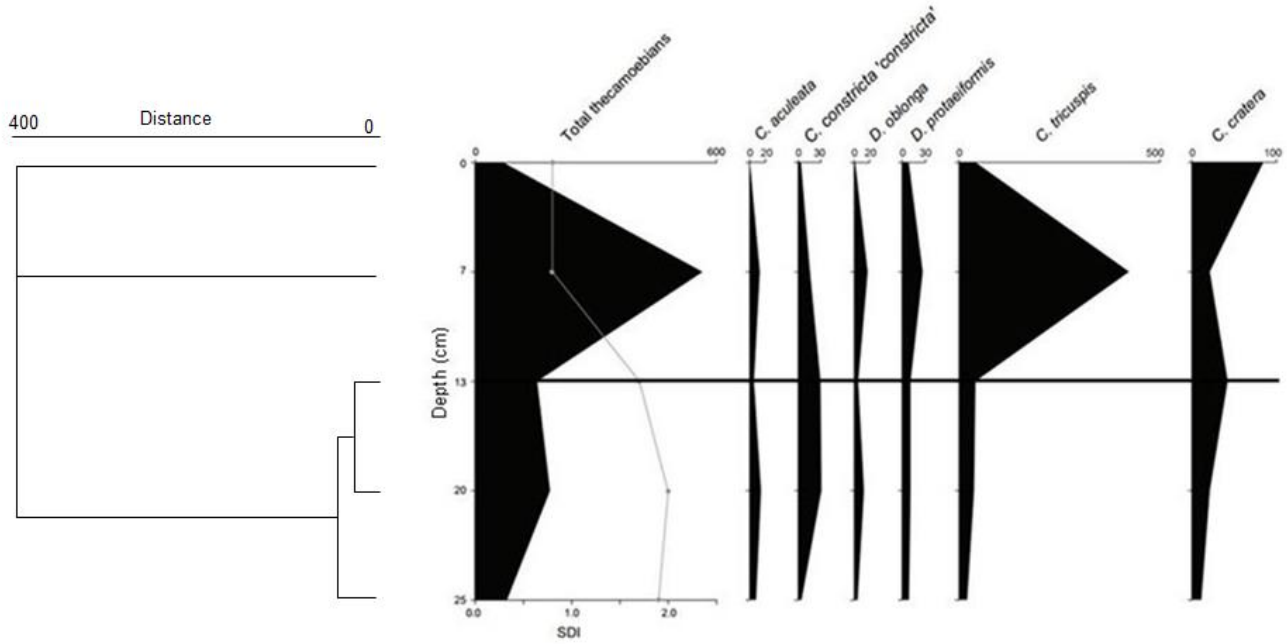


Figure 3.15: Protozoans per ml in Chem-Core with CCA. Note the decrease in total thecamoebians at the surface sediments and the increase in *Codonella cratera* Leidy. The black line at 13 cm represents the rise in *Ambrosia* and other non-arboreal pollen (NAP). SDI is plotted with total thecamoebians per ml.

3.5 Conclusions

Surface grabs and recent sediment cores taken from Sluice Pond exhibit exceptional dinocyst preservation in the deep basin of this urban pond. Initial assessments of protozoan communities depict assemblage changes reflecting anthropogenic impact. The presence of ciliates such as *Codonella cratera* in sediments from the Rio Grande Reservoir, Brazil examined by Barbieri and Orlandi (1989) determined that *C. cratera* increases in relation to anthropogenically impacted, anoxic, and muddy bottom waters and is often present in hypereutrophic lakes. A similar relationship has been observed in Sluice Pond where the presence of *C. cratera* increases in the deep, anoxic basin along with the *Cucurbitella tricuspis* (a thecamoebian with a planktonic phase).

This chapter examined the spatial and temporal response of dinocysts and protozoans to human development and increased heavy metal loading in this partially meromictic lake.

Increased industrialization and population growth has negatively affected freshwater organisms, as indicated by a decrease in protozoan and dinocyst communities at Chem-Core. As populations expanded and algal blooms became frequent, the pond was enriched with herbicides. This is the likely cause of low dinocyst populations at the surface sediments. The presence of high populations of *Peridinium willei*, and the ciliate *Codonella cratera* in heavy metal impacted sediments indicates extremely hostile, anoxic and eutrophic conditions. Water chemistry measured confirms anoxia at the basin, which corroborates with the rich *C. cratera* assemblage. Low abundances of *Difflugia oblonga* in the deep basin suggest that this is a function of oxygen availability. Likewise, the absence of *Arcella vulgaris* in heavy metal impacted sediments suggests low DO plays a role. Low abundances of dinocysts at the pond's margins reflects dilution as a result of terrigenous flux, mass wasting and oxygenation.

The lack of dinocysts and thecamoebians in the woody particulate material indicates rapid burial in SP Cores 2 and 3 during clear-cutting in the 17th century. While this allowed for the preservation of exceptional cysts and thecae, it affected total abundances, particularly the thecamoebians where centropyxids were present due to hostile conditions.

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Chapter 4:

General conclusions

Sluice Pond is a prime location for studying freshwater dinoflagellates and their cysts. Low dissolved oxygen (DO) in the partially meromictic deep basin of this urban pond as well as rapid sedimentation and high biochemical oxygen demand (BOD) attributed to anthropogenic impact over the last few centuries resulted in very high dinocyst concentrations.. This includes several unknown morphotypes as well as several specimens of cellulosic thecae of the small, thick-walled genus *Parvodinium* sp. rival the record in the well-known and intensely studied Crawford Lake site (Burden et al. 1986; McAndrews and Boyko-Diakonow 1989; Ekdahl et al. 2007; Krueger 2012; McCarthy and Krueger 2013). The cyst diversity is even greater including maximum cyst abundances rising markedly to >200,000 cysts/ml in ragweed-rich sediments of the marginal core. Compared to Crawford Lake where a total of four cyst morphotypes were found, attributed to *Peridinium willei* Huitfeld Kaas, *Peridinium volzii* Lemmerman, *Peridinium wisconsinense* Eddy and *Parvodinium inconspicuum* (Lemmerman) Carty, Sluice Pond contains twelve cyst morphotypes. Large, ornamented cysts with a visible paracingulum and paratabulation are attributed to *Peridinium gatunense* Nygaard and are are common in Holocene sediments from the deep basin core and throughout the lakebed since settlement by Puritans in the 17th centruy, but they have not previously been reported in paleolimnological studies, although their cysts are known from limnological studies in Lake Kinneret (Viner-Mozzini et al. 2003; Zohary et al. 2012). The spatial and temporal distribution of these cysts suggests that taphonomy plays an important role in cyst preservation as cysts of *Peridinium willei* are common in shallower water environments, suggesting that they are less sensitive to oxidation.

Stratification and resulting bottom water anoxia in the early Holocene prevented benthic protozoans from colonizing this pond, but allowed dinoflagellates to flourish, resulting in assemblages rich in *Peridinium wisconsinense* that characterized oligotrophic environments. The absence of protozoans in the early Holocene is unique to Sluice Pond as normally ubiquitous thecamoebians such as centropyxids (e.g. *Centropyxis aculeata* Ehrenberg, *Centropyxis constricta* Ehrenberg) are present in harsh and hostile conditions such as: following ice sheet retreat, cold temperatures, low nutrient levels and heavy metal contamination (Decloitre 1956; Schonborn 1984; McCarthy et al. 1995; Patterson et al. 1996; Reinhardt et al. 1998; Patterson and Kumar 2002). As a result of the mid-Holocene drought, protozoans flourished as lower lake

levels and mixing of the water column promoted oxygenation of the bottom waters. Initial colonizers such as *Centropyxis aculeata* and *Difflugia oblonga* Ehrenberg began to increase while the planktonic ciliate *Codonella cratera* Leidy decreased. This decrease in *C.cratera* is attributed to oxygenation of the bottom waters as this planktonic ciliate prefers anoxia (Barbieri and Orlandi 1989; Danesh et al. 2013). Dinocysts attributed to *Peridinium gatunense* and *Perdinium willei* also increased as a result of the breakdown of stratification. PAST analysis confirms microfossil assemblage changes at 5,000, 2,000 and 300 cal BP.

The presence of the planktonic ciliate *C. cratera* beginning in the mid-Holocene indicates a response to anoxia as a result of the development of warmer and wetter conditions following cool and dry conditions of the early Holocene. This climate change resulted in the deepening of Sluice Pond, causing low DO in the basin. Studies by Barbieri and Orlandi (1989), Danesh et al. (2013) and Volik (2014) only record the presence of *C. cratera* in anthropogenically impacted sediments. The unique basin geometry and affinity for meromixis allowed this ciliate to flourish well before human impact. Its high abundance below the chemocline indicates that this ciliate can escape unfavourable or harsh environments due to its planktonic nature. Studies of Sluice Pond reveal that the largely ignored ciliate *C.cratera* should be examined with thecamoebians to better understand paleo-environments and anthropogenically impacted sediments. Protozoan populations record a trend toward decreasing DO as water levels rose throughout New England as a result of climate change, and as a result of human settlement in the upland.

Future research on Sluice Pond includes extensive diatom study, germination of dinocysts, heavy metal toxicology reports and more radiocarbon dates in the marginal core. Diatoms have been well studied for several decades and their presence would corroborate with less well researched dinoflagellate cysts. Diatoms could further shed some light on the dynamic climate changes in the Holocene. Additional research includes the germination of unknown and potentially new dinoflagellate cysts. The tentative names given to cysts in this study can be improved. Heavy metal toxicology studies of the surface sediments and their effects on protozoans in particular would be useful in further cememting protozoans as proxies for severe anthropogenic impact.

Appendix 1a: Dinocyst raw data for Core SP09 KC2

Depth (cm)	Age (cal BP)	Lycopodium	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total	SDI
0	0.0	32	25	-	-	4	2	1	-	-	-	32	0.7
10	159.5	15	6	-	3	3	75	-	-	-	-	87	0.5
20	365.0	17	49	-	11	20	19	-	1	-	-	100	1.3
30	580.6	35	34	1	16	18	15	-	-	-	-	84	1.4
40	733.0	17	27	3	8	29	8	-	-	-	-	75	1.3
50	1037.4	27	24	-	25	19	6	-	1	-	-	75	1.3
60	1276.6	15	32	-	18	14	31	-	-	-	-	95	1.3
70	1521.7	15	44	-	5	19	17	1	-	-	-	86	1.2
80	1771.9	18	46	-	2	10	2	-	-	-	-	60	0.7
90	2026.3	19	42	1	4	6	4	1	1	-	-	59	1.0
100	2284.2	18	36	-	9	10	19	1	-	-	-	75	1.3
110	2544.7	16	117	-	36	32	48	-	-	-	-	233	1.2
120	2807.3	15	47	-	13	33	13	-	-	1	-	107	1.2
130	3071.1	22	32	-	11	23	9	-	-	-	-	75	1.3
140	3335.8	16	42	-	18	7	13	-	-	-	-	80	1.2
150	3600.6	57	28	-	7	2	31	-	7	-	-	75	1.2
160	3865.0	20	28	-	3	12	14	1	-	-	2	60	1.3
170	4128.7	15	73	-	18	10	14	1	-	-	-	116	1.1
180	4391.3	15	78	1	11	4	5	-	-	-	-	99	0.8
191	4678.3	15	54	-	6	5	7	-	-	-	2	74	0.9
201	4937.2	18	23	-	3	8	41	-	-	-	-	75	1.0
211	5194.0	15	20	-	-	10	43	-	-	-	-	73	0.9
221	5448.4	21	18	-	5	4	47	1	-	-	-	75	1.0
231	5700.4	20	25	-	-	10	44	-	-	-	-	79	0.9
241	5949.7	17	20	-	4	5	28	-	-	-	-	57	1.1
251	6196.3	15	19	-	1	2	67	-	-	-	-	89	0.7
261	6440.2	15	15	-	-	3	59	-	-	-	-	77	0.7
271	6681.4	20	10	-	2	2	61	-	-	-	-	75	0.6
281.5	6931.8	20	9	-	-	16	101	-	-	-	-	126	0.6
291	7156.1	20	14	-	5	1	57	-	-	-	-	77	0.7
301	7389.9	24	27	-	3	7	41	-	-	-	-	78	1.0
311	7621.6	22	35	-	23	36	25	-	-	-	-	119	1.4
331	8079.8	19	21	-	1	2	55	-	-	-	-	79	0.7
341	8307.1	15	20	-	-	8	78	-	-	-	-	106	0.7
351	8533.6	15	14	-	5	2	62	-	-	-	-	83	0.8
370.5	8975.0	15	26	-	4	9	20	-	-	-	-	59	1.2
380.5	9202.2	17	5	1	1	5	63	-	-	-	-	75	0.6
390.5	9430.8	64	13	-	4	17	46	-	-	-	-	80	1.1
402	9696.2	38	26	-	20	28	-	-	-	-	1	75	1.1
410.5	9894.7	38	38	-	3	30	2	2	-	-	-	75	1.0
430.5	10372.6	30	16	-	17	32	10	-	-	-	-	75	1.3
440.5	10618.8	15	22	-	4	11	43	-	-	-	-	80	1.1
457.5	11051.5	77	24	-	11	12	-	-	-	-	-	47	1.0

Appendix 1b: Dinocysts per ml in Core SP09 KC2

Depth (cm)	Lycopodium	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total
0	32	3391	-	-	543	271	136	-	-	-	4340
10	15	1736	-	868	868	21700	-	-	-	-	25172
20	17	12509	-	2808	5106	4851	-	255	-	-	25529
30	35	4216	124	1984	2232	1860	-	-	-	-	10416
40	17	6893	766	2042	7404	2042	-	-	-	-	19147
50	27	3858	-	4019	3054	964	-	161	-	-	12056
60	15	9259	-	5208	4919	8969	-	-	-	-	28355
70	15	12731	-	1447	5497	4919	-	289	-	-	24883
80	18	11091	-	482	2411	482	-	-	-	-	14467
90	19	9594	228	914	1371	914	228	228	-	-	13477
100	18	8680	-	2170	2411	4581	241	-	-	-	18083
110	16	31736	-	9765	8680	13020	-	-	-	-	63201
120	15	13599	-	3761	9548	3761	-	-	289	-	30959
130	22	6313	-	2170	4537	1775	-	-	-	-	14795
140	16	11393	-	4883	1899	3526	-	-	-	-	21700
150	48	2132	-	533	152	2360	-	533	-	-	5711
160	20	6076	-	651	2604	3038	217	-	-	434	12152
170	15	21121	-	5208	2893	4051	289	-	-	-	33563
180	15	22568	289	3183	1157	1447	-	-	-	-	28644
191	15	15624	-	1736	1447	2025	-	-	-	579	21411
201	18	5546	-	723	1929	9886	-	-	-	-	18083
211	15	5787	-	-	2893	12441	-	-	-	-	21121
221	21	3720	-	1033	827	9713	207	-	-	-	15500
231	18	5425	-	-	2170	9548	-	-	-	-	17143
241	17	5106	-	1021	1276	7148	-	-	-	-	14552
251	15	5497	-	289	579	19385	-	-	-	-	25751
261	15	4340	-	-	868	17071	-	-	-	-	22279
271	20	2170	-	434	434	13237	-	-	-	-	16275
282	20	1953	-	-	3472	21917	-	-	-	-	27342
291	20	3038	-	1085	217	12369	-	-	-	-	16709
301	24	4883	-	543	1266	7414	-	-	-	-	14105
311	22	6905	-	4537	7102	4932	-	-	-	-	23475
331	19	4797	-	228	457	12563	-	-	-	-	18045
341	15	5787	-	-	2315	22568	-	-	-	-	30669
351	15	4051	-	1447	579	17939	-	-	-	-	24015
371	15	7523	-	1157	2604	5787	-	-	-	-	17071
381	17	1276	255	255	1276	16084	-	-	-	-	19147
391	64	882	-	271	1153	3119	-	-	-	-	5425
402	38	2969	-	2284	3198	-	-	-	-	124	8576
411	38	4340	-	228	3426	228	343	-	-	-	8566
431	30	2315	-	2459	4629	1447	-	-	-	-	10850
441	15	6365	-	1157	3183	12441	-	-	-	-	23147
458	77	1353	-	620	676	-	-	-	-	-	2649

Appendix 1c: Dinocyst percent abundance and percent error in Core SP09 KC2

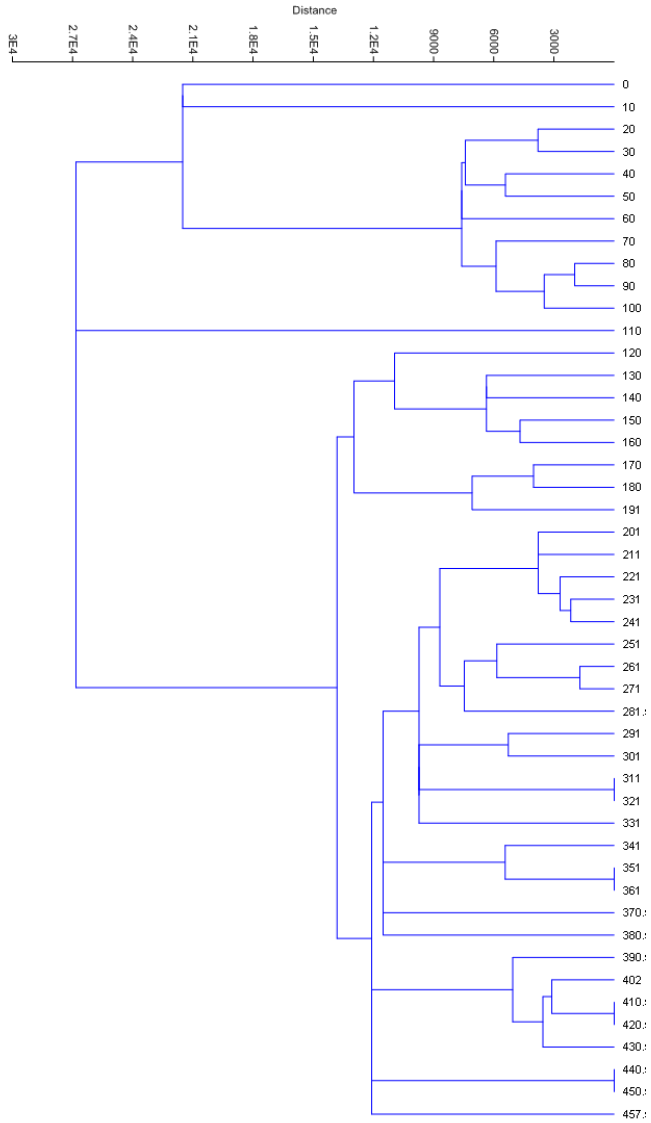
	P. gatunense		*P. limbatum*		*P. volzii*		*P. willei*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	78.1250	1.4324	-	-	-	-	12.5000	1.1459
10	10.1695	0.7712	-	-	5.0847	0.5606	5.0847	0.5606
20	49.0000	0.9798	-	-	11.0000	0.6133	20.0000	0.7840
30	40.4762	1.0497	1.1905	0.2319	19.0476	0.8398	21.4286	0.8775
40	36.0000	1.0863	4.0000	0.4435	10.6667	0.6986	38.6667	1.1022
50	32.0000	1.0557	-	-	33.3333	1.0669	25.3333	0.9843
60	33.6842	0.9504	-	-	18.9474	0.7880	14.7368	0.7128
70	51.1628	1.0565	-	-	5.8140	0.4946	22.0930	0.8768
80	76.6667	1.0702	-	-	3.3333	0.4542	16.6667	0.9430
90	71.1864	1.1557	1.6949	0.3294	6.7797	0.6415	10.1695	0.7712
100	48.0000	1.1300	-	-	12.0000	0.7354	13.3333	0.7693
110	50.2146	0.6420	-	-	15.4506	0.4641	13.7339	0.4420
120	43.9252	0.9404	-	-	12.1495	0.6190	30.8411	0.8751
130	42.6667	1.1194	-	-	14.6667	0.8007	30.6667	1.0436
140	51.8519	1.0881	-	-	22.2222	0.9054	8.6420	0.6119
150	37.3333	1.0947	-	-	9.3333	0.6584	2.6667	0.3646
160	46.6667	1.2624	-	-	5.0000	0.5515	20.0000	1.0121
170	62.9310	0.8790	-	-	15.5172	0.6589	8.6207	0.5108
180	78.7879	0.8053	1.0101	0.1970	11.1111	0.6191	4.0404	0.3879
191	72.9730	1.0119	-	-	8.1081	0.6219	6.7568	0.5719
201	30.6667	1.0435	-	-	4.0000	0.4434	10.6667	1.0667
211	27.3973	1.0231	-	-	-	-	13.6986	0.7888
221	24.0000	0.9666	-	-	6.6667	0.5645	5.3333	0.5085
231	31.6456	1.0256	-	-	-	-	12.6582	0.7332
241	35.0877	1.2390	-	-	7.0175	0.6631	8.7719	0.7344
251	21.3483	0.8513	-	-	1.1236	0.2190	2.2472	0.3079
261	19.4805	0.8846	-	-	-	-	3.8961	0.4322
271	13.3333	0.7693	-	-	2.6667	0.3646	2.6667	0.3646
281.5	7.1429	0.4497	-	-	-	-	12.6984	0.5814
291	18.1818	0.8615	-	-	6.4935	0.5504	1.2987	0.2529
301	34.6154	1.0558	-	-	3.2787	0.4469	3.8462	0.4268
311	29.4118	0.8187	-	-	19.3277	0.7095	30.2521	0.8253
331	26.5823	0.9742	-	-	1.2658	0.2465	2.5316	0.3464
341	18.8679	0.7448	-	-	-	-	7.5472	0.5029
351	16.8675	0.8056	-	-	6.0241	0.5119	2.4096	0.3299
370.5	44.0678	1.2668	-	-	6.7797	0.6415	15.2542	0.9175
380.5	6.6667	0.5645	1.3333	0.2596	1.3333	0.2596	6.6667	0.5645
390.5	16.2500	0.8084	-	-	5.0000	0.4776	21.2500	0.8964
402	34.6667	1.0771	-	-	26.6667	1.0008	37.3333	1.0947
410.5	60.0000	1.2396	-	-	2.6667	0.3646	40.0000	1.1087
430.5	50.6667	1.1315	-	-	22.6667	0.9476	42.6667	1.1194
440.5	27.5000	0.9785	-	-	5.0000	0.4776	13.7500	0.7546
457.5	51.0638	1.4292	-	-	23.4043	1.2105	25.5319	1.2466

P. wisconsinense		Cyst type G		Cyst type J		Cyst type K		Cyst type L		
%A	E	%A	E	%A	E	%A	E	%A	E	Raw data total
6.2500	0.8387	3.1250	0.6029	-	-	-	-	-	-	32
84.7458	0.9175	-	-	-	-	-	-	-	-	87
19.0000	0.7689	-	-	1.0000	19.5018	-	-	-	-	100
17.8571	0.8190	-	-	-	-	-	-	-	-	84
10.6667	0.6986	-	-	-	-	-	-	-	-	75
8.0000	0.6140	-	-	1.6393	31.8667	-	-	-	-	75
32.6316	0.9428	-	-	-	-	-	-	-	-	95
19.7674	0.8417	1.1628	0.2266	-	-	-	-	-	-	86
3.3333	0.4542	-	-	-	-	-	-	-	-	60
6.7797	0.6415	1.6949	0.3294	1.6949	32.9376	-	-	-	-	59
25.3333	0.9843	1.3000	2.5900	-	-	-	-	-	-	75
20.6009	0.5193	-	-	-	-	-	-	-	-	233
12.1495	0.6190	-	-	-	-	0.9346	0.1823	-	-	107
12.0000	0.7355	-	-	-	-	-	-	-	-	75
16.0494	0.7994	-	-	-	-	-	-	-	-	80
41.3333	1.1145	-	-	9.3333	0.6584	-	-	-	-	75
23.3333	1.0702	1.6667	0.3239	-	-	-	-	3.3333	45.4212	60
12.0690	0.5928	0.8621	0.1682	-	-	-	-	-	-	116
5.0505	0.4314	-	-	-	-	-	-	-	-	99
9.4595	0.6668	-	-	-	-	-	-	2.7027	36.9479	74
54.6667	1.2667	-	-	-	-	-	-	-	-	75
58.9041	1.1287	-	-	-	-	-	-	-	-	73
62.6667	1.0947	1.3333	0.2596	-	-	-	-	-	-	75
55.6962	1.0954	-	-	-	-	-	-	-	-	79
49.1228	1.2978	-	-	-	-	-	-	-	-	57
75.2809	0.8962	-	-	-	-	-	-	-	-	89
74.6032	1.0749	-	-	-	-	-	-	-	-	77
81.3333	0.8818	-	-	-	-	-	-	-	-	75
76.6234	0.9453	-	-	-	-	-	-	-	-	126
74.0260	0.9794	-	-	-	-	-	-	-	-	77
89.7436	0.6343	-	-	-	-	-	-	-	-	78
21.0084	0.7319	-	-	-	-	-	-	-	-	119
69.6203	1.0142	-	-	-	-	-	-	-	-	79
73.5849	0.8393	-	-	-	-	-	-	-	-	106
74.6988	0.9353	-	-	-	-	-	-	-	-	83
33.8983	1.2079	-	-	-	-	-	-	-	-	59
84.0000	0.8297	-	-	-	-	-	-	-	-	75
57.5000	1.0833	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	1.6949	32.9376	75
2.6667	0.3646	4.0000	0.4435	-	-	-	-	-	-	75
13.3333	0.7693	-	-	-	-	-	-	-	-	75
53.7500	1.0926	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	47

Appendix 1d: Age against depth, absolute abundances (concentrations) and relative abundances (percentages) of dinocysts.

		cysts/ml	%	cysts/ml	%	cysts/ml	%	cysts/ml	%
Depth	Age	*P. gatunense*	*P. gatunense*	*P. volzii*	*P. volzii*	*P. willei*	*P. willei*	*P. wisconsinense*	*P. wisconsinense*
0	0	3391	78.1 +/- 1.4	-	-	543	12.5 +/- 1.1	271	6.3 +/- 0.8
10	160	1736	10.2 +/- 0.8	868	5.1+/-0.6	868	5.1 +/- 0.6	21,700	84.7 +/- 0.9
20	365	12,509	49.0 +/- 1.0	2808	11.0 +/- 0.6	5106	20.0 +/- 0.8	4851	19.0 +/- 0.8
30	581	4,216	40.4 +/- 1.0	1984	19.0 +/- 0.8	2232	21.4 +/- 0.9	1860	17.9 +/- 0.8
40	733	6893	36.0 +/- 1.1	2042	10.7 +/- 0.7	7404	38.7 +/- 0.7	2042	10.7 +/- 0.7
50	1037.4	3857	32.0 +/- 1.1	4018	33.3 +/- 0.7	3054	25.3 +/- 0.9	964	8.0 +/- 0.6
60	1276.6	9259	33.7 +/- 1.0	5208	18.9 +/- 0.8	4919	14.7 +/- 0.7	8969	32.6 +/- 0.9
70	1521.7	12730	19.8 +/- 1.0	1447	5.8 +/- 0.5	5497	22.1 +/- 0.9	4918	19.8 +/- 0.8
80	1771.9	11091	76.7 +/- 1.1	482	3.3 +/- 0.5	2411	16.7 +/- 0.9	482	3.3 +/- 0.5
90	2026.3	9594	71.2 +/- 1.2	914	6.8 +/- 0.6	1371	10.2 +/- 0.8	914	6.8 +/- 0.6
100	2284.2	8680	48.0 +/- 1.1	2170	12.0 +/- 0.7	2411	13.3 +/- 0.8	4581	25.3 +/- 1.1
110	2544.7	31736	50.2 +/- 0.6	9765	15.5 +/- 0.4	8680	13.7 +/- 0.4	13020	20.6 +/- 0.5
120	2807.3	13599	43.9 +/- 0.9	3761	12.1 +/ 0.6	9548	30.8 +/- 0.9	3761	12.1 +/- 0.6
130	3071.1	6312	42.7 +/- 1.1	2170	14.7 +/- 0.8	4537	30.7 +/- 1.0	1775	12.0 +/- 0.7
140	3335.8	11393	51.9 +/- 1.1	4883	22.2 +/- 0.9	1899	8.6 +/- 0.6	3526	16.0 +/- 0.8
150	3600.6	2132	37.3 +/- 1.1	533	9.3 +/- 0.7	152	2.7 +/- 0.4	2360	41.3 +/- 1.1
160	3865.0	6076	46.7 +/- 1.3	651	5.0 +/- 0.6	2604	20.0 +/- 1.0	3038	23.3 +/- 1.1
170	4128.7	21121	62.9 +/- 0.9	5208	15.5 +/- 0.7	2893	8.6 +/- 0.5	4051	12.2 +/- 0.6
180	4391.3	22568	78.8 +/- 0.8	3183	11.1 +/- 0.6	1157	4.0 +/- 0.4	1447	5.1 +/- 0.4
191	4678.3	15626	73.0 +/- 1.0	1736	8.1 +/- 0.6	1447	6.8 +/- 0.5	2025	9.5 +/- 0.7
201	4937.2	91622	30.7 +/- 1.0	4822	4.0 +/- 0.4	22423	10.7 +/- 1.1	134549	54.7 +/- 1.3
211	5194.0	5787	27.4 +/- 1.0	-	-	2893	13.7 +/- 0.8	12441	58.9 +/- 1.1
221	5448.4	3720	24.0 +/- 1.0	1033	6.7 +/- 0.7	827	5.3 +/- 0.5	9713	62.7 +/- 1.1
231	5700.4	5425	31.6 +/- 1.0	-	-	2170	12.7 +/- 0.7	9548	55.7 +/- 1.1
241	5949.7	5106	35.1 +/- 1.2	1021	7.0 +/- 0.7	1276	8.8 +/- 0.7	7148	49.1 +/- 1.3
251	6196.3	5497	21.3 +/- 0.9	289	1.1 +/- 0.2	579	2.2 +/- 0.3	19385	75.3 +/- 0.9
261	6440.2	4883	19.5 +/- 0.9	-	-	543	3.9 +/- 0.4	7414	74.6 +/- 1.1
271	6681.4	2170	13.3 +/- 0.8	434	2.7 +/- 0.4	434	2.7 +/- 0.4	13237	81.3 +/- 0.9
281.5	6931.8	1953	7.1 +/- 0.5	-	-	3472	12.7 +/- 0.6	21917	80.1 +/- 0.7
291	7156.1	3038	18.8 +/- 0.9	1085	6.5 +/- 0.6	217	1.3 +/- 0.3	12369	74.0 +/- 1.0
301	7389.9	4960	34.6 +/- 0.1	413	3.3 +/- 0.4	413	3.8 +/- 0.4	6820	89.7 +/- 0.6
311	7621.6	6905	29.4 +/- 0.8	4537	19.3 +/- 0.7	7101	30.3 +/- 0.8	4932	21.0 +/- 0.7
331	8079.8	4797	26.6 +/- 1.0	228	1.3 +/- 0.2	457	2.5 +/- 0.3	12563	69.6 +/- 1.0
341	8307.1	5786	18.7 +/- 0.7	-	-	2315	7.5 +/- 0.5	22568	73.6 +/- 0.8
351	8533.6	4051	16.9 +/- 0.8	1447	6.0 +/- 0.5	579	2.4 +/- 0.3	17939	74.7 +/- 0.9
370.5	8975.0	7523	44.1 +/- 1.3	1157	6.8 +/- 0.6	2604	15.3 +/- 0.9	5787	33.0 +/- 1.2
380.5	9202.2	1276	6.7 +/- 0.5	255	1.3 +/- 0.3	1276	6.7 +/- 0.5	16083	84.0 +/- 0.8
390.5	9430.8	881	16.3+/- 0.8	271	5.0 +/- 0.5	1153	21.3 +/- 0.9	3120	57.5 +/- 1.1
402	9696.2	2969	34.7 +/- 1.1	2284	26.7 +/- 1.0	3198	37.3 +/- 1.1	-	-
410.5	9894.7	4340	60.0 +/- 1.2	228	2.7 +/- 0.4	3426	40.0 +/- 1.1	228	2.7 +/- 0.4
430.5	10372.6	2314	21.3 +/- 0.9	2459	22.7 +/- 0.9	4629	42.7 +/- 1.1	1447	13.3 +/- 0.1
440.5	10618.8	6365	24.5 +/- 1.0	1157	5.0 +/- 0.5	3183	13.8 +/- 0.8	12441	53.8 +/- 1.1
457.5	11051.5	1353	51.1 +/- 1.4	620	23.4 +/- 1.2	676	25.5 +/- 1.2	-	-

Appendix 1e: Constrained cluster analysis for SP09 KC2



Appendix 2a: Dinocyst raw data for Core SP07 PC4

Depth (cm)	Lycopodium	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type J	Cyst type K	Total	SDI
0	19	7	-	4	8	2	-	-	21	1.3
10	60	12	-	3	13	83	-	-	111	0.8
20	45	1	-	8	2	74	-	-	85	0.5
40	21	9	-	13	34	23	-	-	80	1.3
50	48	9	-	35	28	8	-	-	80	1.2
70	66	7	-	42	22	7	-	2	80	1.2
80	50	8	1	15	45	12	-	-	81	1.2
90	42	11	-	18	30	27	-	-	86	1.3
100	43	16	-	5	9	53	-	-	83	1.0
110	28	11	-	7	17	46	-	-	81	1.1
130	21	14	-	7	12	48	-	-	81	1.1
140	17	3	-	5	5	67	-	-	80	0.6
160	49	9	-	5	14	51	1	-	80	1.1
170	20	12	1	3	7	57	-	-	80	0.9
190	56	7	-	5	4	66	-	-	82	0.7

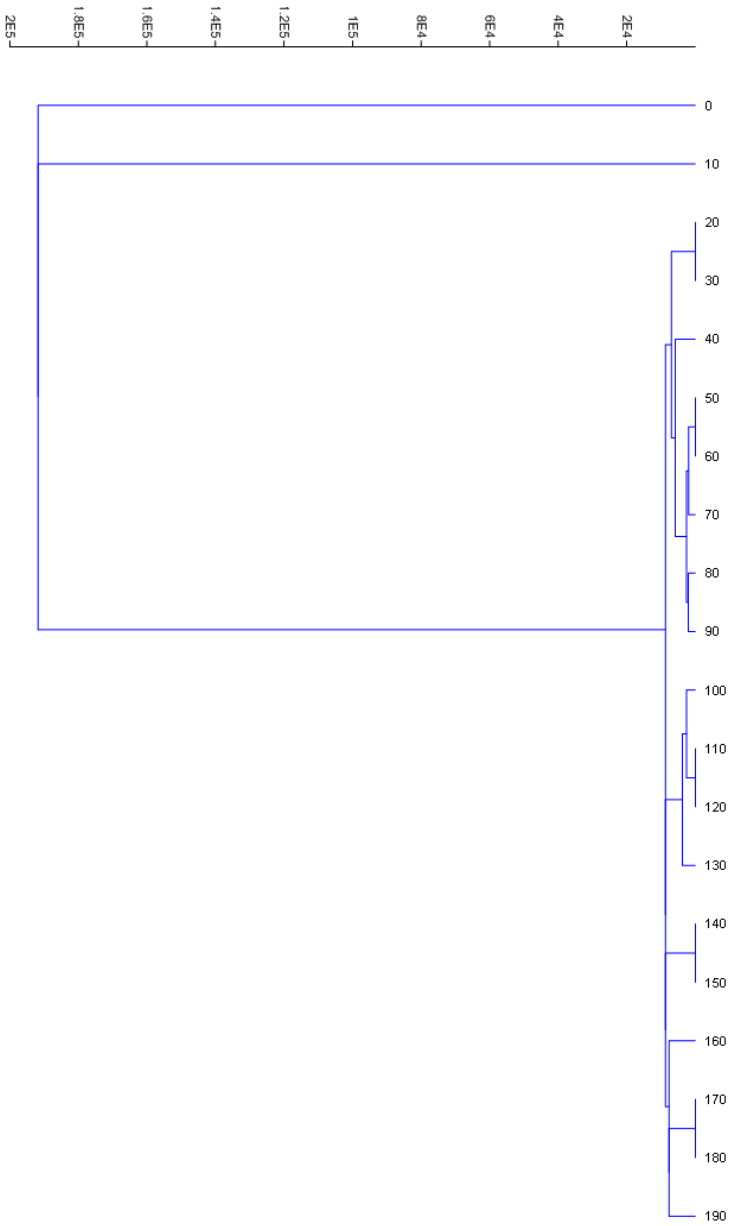
Appendix 2b: Dinocysts per ml in Core SP07 PC4

Depth (cm)	Lycopodium	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type J	Cyst type K	Total
0	19	1599	-	914	1827	10	-	-	2751
10	60	35009	-	8752	940	190979	-	-	235681
20	45	96	-	772	193	416	-	-	1381
40	21	1860	-	2687	7027	37	-	-	9750
50	48	814	-	3165	2532	11	-	-	5707
70	66	460	-	1644	1447	18	-	132	3241
80	50	694	87	1302	3906	40	-	-	5335
90	42	1137	-	1860	3100	63	-	-	5023
100	43	1615	-	505	908	456	-	-	1869
110	28	1705	-	1085	2635	184	-	-	3904
130	21	2893	-	1447	2480	144	-	-	4071
140	17	766	-	1276	1276	228	-	-	2781
160	48	814	-	452	1266	490	90	-	2298
170	20	2604	217	651	1519	380	-	-	2767
190	56	543	-	388	310	739	-	-	1437

Appendix 2c: Dinocyst percent abundance and percent error in Core SP07 PC4

	P. gatunense		*P. limbatum*		*P. volzii*		*P. willei*		*P. wisconsinense*		Cyst type J		Cyst type K		
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**Raw data total**
0	33.3333	2.0162	-	-	19.0476	1.6795	38.0952	2.0770	9.5238	1.2555	-	-	-	-	21
10	10.8108	0.5777	-	-	2.7027	0.3017	11.7117	0.5982	74.7748	0.8080	-	-	-	-	111
20	1.1765	0.2292	-	-	9.4118	0.6208	2.3529	0.3222	87.0588	0.7136	-	-	-	-	85
40	11.2500	0.6924	-	-	15.0000	0.7825	42.5000	1.0833	28.7500	0.9918	-	-	-	-	80
50	11.2500	0.6924	-	-	43.7500	1.0871	35.0000	1.0452	10.0000	0.6574	-	-	-	-	80
70	8.7500	0.6192	-	-	31.2500	1.0157	27.5000	0.9785	8.7500	0.6192	-	-	2.5000	0.3421	80
80	9.8765	0.6497	1.2346	0.2405	18.5185	0.8460	55.5556	1.0821	14.8148	0.7736	-	-	-	-	81
90	12.7907	0.7059	-	-	20.9302	0.8598	34.8837	1.0073	31.3953	0.9809	-	-	-	-	86
100	19.2771	0.8487	-	-	6.0241	0.5119	10.8434	0.6689	63.8554	1.0336	-	-	-	-	83
110	13.5802	0.7461	-	-	8.6420	0.6119	20.9877	0.8868	56.7901	1.0788	-	-	-	-	81
130	17.2840	0.8234	-	-	8.6420	0.6119	14.8148	0.7736	59.2593	1.0701	-	-	-	-	81
140	3.7500	0.4163	-	-	6.2500	0.5304	6.2500	0.5304	83.7500	0.8084	-	-	-	-	80
160	11.2500	0.6924	-	-	6.2500	0.5304	17.5000	0.8326	63.7500	1.0534	1.2500	0.2435	-	-	80
170	15.0000	0.7825	1.2500	0.2435	3.7500	0.4163	8.7500	0.6192	71.2500	0.9918	-	-	-	-	80
190	8.5366	0.6048	-	-	6.0976	0.5179	4.8780	0.4662	80.4878	0.8578	-	-	-	-	82

Appendix 2d: Constrained cluster analysis for SP07 PC4



Appendix 3a: Dinocyst raw data for SP Core 2

Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Total	SDI
0	68	1	1	-	1	17	2	-	-	-	1	23	1.2
5	123	-	-	35	2	7	2	-	-	2	-	48	1.2
10	70	-	1	2	-	2	-	1	-	-	-	5	1.3
15	78	-	-	-	-	-	-	-	-	-	-	0	N/A
20	9	-	-	-	-	-	-	-	-	-	-	0	N/A
25	101	-	1	3	-	1	1	-	-	1	-	7	1.5
30	73	-	2	1	1	-	-	-	-	-	-	2	0.7
35	160	-	1	-	1	-	5	-	-	-	-	6	0.5
40	231	-	3	13	5	1	20	-	-	2	-	41	1.3
45	104	-	-	3	-	-	3	-	-	-	-	6	0.7
50	35	-	-	-	-	1	1	-	-	-	-	3	1.1
55	94	-	-	14	3	-	31	-	2	-	-	50	1.0
60	105	-	1	1	-	1	5	-	-	-	-	7	1.1
74	16	-	-	-	-	-	-	-	-	-	-	0	N/A

Appendix 3b: Dinocysts per ml in SP Core 2

Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Total
0	68	64	64	-	64	1085	128	-	-	-	64	1468
5	123	-	35	1423	81	285	81	-	-	81	-	1987
10	70	-	62	124	-	124	-	62	-	-	-	372
15	78	-	-	-	-	-	-	-	-	-	-	-
20	9	-	-	-	-	-	-	-	-	-	-	-
25	101	-	43	149	-	50	50	-	-	50	-	340
30	73	-	119	59	59	-	-	-	-	-	-	238
35	160	-	31	-	31	-	156	-	-	-	-	219
40	231	-	56	244	94	19	376	-	-	38	-	827
45	104	-	-	144	-	-	144	-	-	-	-	288
50	35	-	-	-	-	124	124	-	106	-	-	354
55	94	-	-	745	160	106	1862	-	-	-	-	2872
60	105	-	41	41	-	41	207	-	-	-	-	331
74	16	-	-	-	-	-	-	-	-	-	-	-

Appendix 3c: Dinocyst percent abundance and percent error in SP Core 2

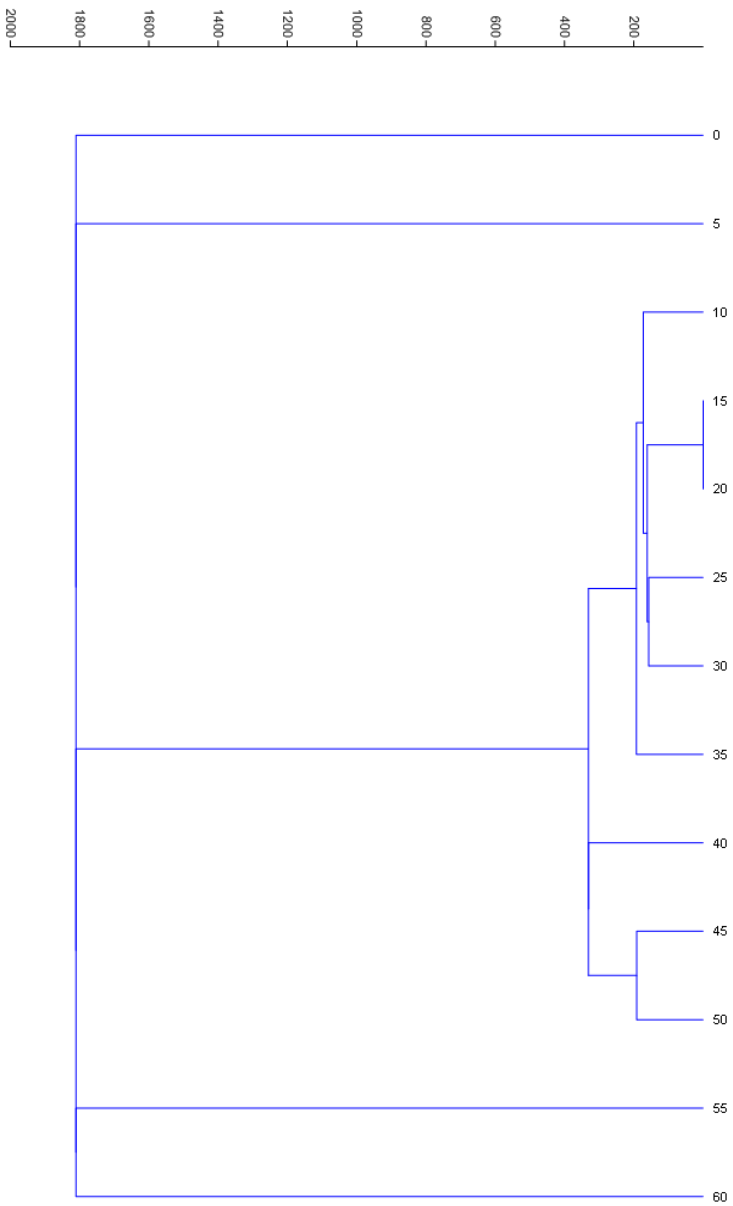
	Chimonodinium spp.		*P. inconspicuum*		*P. gatunense*		*P. volzii*		*P. willei*
Depth (cm)	%A	E	%A	E	%A	E	%A	E	%A
0	4.5455	0.8704	-	-	-	-	4.5455	0.8704	77.2727
5	-	-	-	-	72.9167	1.2572	4.1667	0.5653	14.5833
10	-	-	0.1667	0.2982	40.0000	4.2941	-	-	40.0000
15	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
25	-	-	0.1429	0.2592	50.0000	4.0008	-	-	16.6667
30	-	-	0.5000	0.4900	50.0000	6.9296	50.0000	6.9296	-
35	-	-	0.1429	0.2592	-	-	16.6667	2.9820	-
40	-	-	0.7500	0.1279	31.7073	1.4244	12.1951	1.0017	2.4390
45	-	-	-	-	50.0000	4.0008	-	-	-
50	-	-	-	-	-	-	-	-	50.0000
55	-	-	-	-	28.0000	1.2446	6.0000	0.6583	-
60	-	-	0.0217	0.1011	14.2857	2.5923	-	-	14.2857
74	-	-	-	-	-	-	-	-	-

P. wisconsinense		Cyst type C/D		Cyst type G		Cyst type J		Cyst type K	
%A	E	%A	E	%A	E	%A	E	%A	E
9.0909	1.2013	-	-	-	-	-	-	4.5455	0.8704
4.1667	0.5653	-	-	-	-	4.1667	0.5653	-	-
-	-	20.0000	3.5062	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
16.6667	2.9820	-	-	-	-	1.6667	2.9820	-	-
-	-	-	-	-	-	-	-	-	-
83.3333	2.9820	-	-	-	-	-	-	-	-
48.7805	1.5300	-	-	-	-	4.8780	0.6594	-	-
50.0000	4.0008	-	-	-	-	-	-	-	-
50.0000	6.9296	-	-	-	-	-	-	-	-
62.0000	1.3454	-	-	4.0000	0.5432	-	-	-	-
71.4286	3.3466	-	-	-	-	-	-	-	-
-	-	-	-	-		-	-	-	-

Appendix 3d: Dinocyst theca abundance per ml in SP Core 2

Depth (cm)	*Parvodinium* (?) sp.
0	-
5	41
10	186
20	964
25	99
30	297
35	63
40	113
50	-
55	106
60	83
74	271

Appendix 3e: Constrained cluster analysis for SP Core 2



Appendix 4a: Dinocyst raw data for SP Core 3

Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. limbatum*	*P. volzii*
0	30	-	1	13	-	2
5	136	11	-	38	-	1
10	31	-	-	15	-	11
15	114	-	-	8	-	4
20	108	-	-	4	-	17
25	75	-	-	2	-	-
30	104	-	2	1	-	4
35	301	-	2	6	-	4
40	42	-	-	-	-	7
45	202	-	-	7	-	-
50	55	-	-	8	-	13
55	251	-	1	6	-	4
60	79	-	1	5	-	21
65	221	-	1	39	1	8
70	70	1	1	4	-	1
75	300	-	1	10	-	-
80	250	-	-	3	-	21
85	226	-	1	18	-	10
90	57	-	2	5	-	6
95	291	-	1	21	-	-
100	45	10	-	6	-	14
105	276	-	1	30	1	5
111	120	-	1	4	-	25

P willei	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total	SDI
39	3	-	1	-	-	1	60	1.1
9	3	-	-	-	-	-	62	0.8
22	7	-	-	-	-	-	55	1.3
4	45	-	-	-	-	-	61	0.8
13	8	4	-	-	-	-	46	1.5
-	-	-	-	-	-	-	2	0.0
8	1	-	-	-	-	-	16	1.1
5	6	-	-	-	-	-	23	1.4
3	2	-	-	-	-	-	12	1.0
-	1	-	-	-	-	-	8	0.4
17	5	2	-	-	1	-	46	1.5
1	13	1	-	-	-	-	26	1.2
12	7	1	-	-	-	-	47	1.3
-	5	-	1	1	-	-	56	1.0
3	36	-	-	-	-	-	46	0.7
1	2	-	-	-	-	-	14	0.7
10	4	1	-	-	-	-	39	1.2
7	4	-	-	-	-	-	40	1.2
7	17	-	-	-	-	-	37	1.3
-	11	-	-	-	-	-	33	0.6
15	10	-	-	-	-	-	55	1.6
4	14	-	-	-	-	-	55	1.2
10	9	-	-	-	-	1	50	1.3

Appendix 4b: Dinocysts per ml in SP Core 3

Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total
0	30	-	145	1881	-	289	5642	434	-	145	-	-	145	8680
5	136	404	-	1397	-	37	331	110	-	-	-	-	-	2279
10	31	-	-	2100	-	1540	3080	980	-	-	-	-	-	7700
15	114	-	-	351	-	175	175	1974	-	-	-	-	-	2675
20	108	-	-	161	-	683	522	321	161	-	-	-	-	1849
25	75	-	-	133	-	-	-	-	-	-	-	-	-	133
30	104	-	83	42	-	167	334	42	-	-	-	-	-	668
35	301	-	33	100	-	66	83	100	-	-	-	-	-	382
40	42	-	-	-	-	723	310	207	-	-	-	-	-	1240
45	202	-	-	173	-	-	-	25	-	-	-	-	-	198
50	55	-	-	631	-	1026	1341	395	158	-	-	79	-	3630
55	251	-	20	120	-	80	20	259	20	-	-	-	-	518
60	79	-	55	275	-	1154	659	385	55	-	-	-	-	2582
65	221	-	23	882	23	181	-	113	-	23	23	-	-	1267
70	70	62	62	248	-	62	186	2232	-	-	-	-	-	2852
75	300	-	17	167	-	-	17	33	-	-	-	-	-	233
80	250	-	-	52	-	365	174	69	17	-	-	-	-	677
85	226	-	23	398	-	221	155	88	-	-	-	-	-	886
90	57	-	152	381	-	457	533	1294	-	-	-	-	-	2817
95	291	-	17	361	-	-	-	189	-	-	-	-	-	567
100	45	964	-	579	-	1350	1447	964	-	-	-	-	-	5304
105	276	-	18	543	18	91	72	254	-	-	-	-	-	996
111	120	-	36	145	-	904	362	326	-	-	-	-	36	1808

Appendix 4c: Dinocyst percent abundance and percent error in SP Core 3

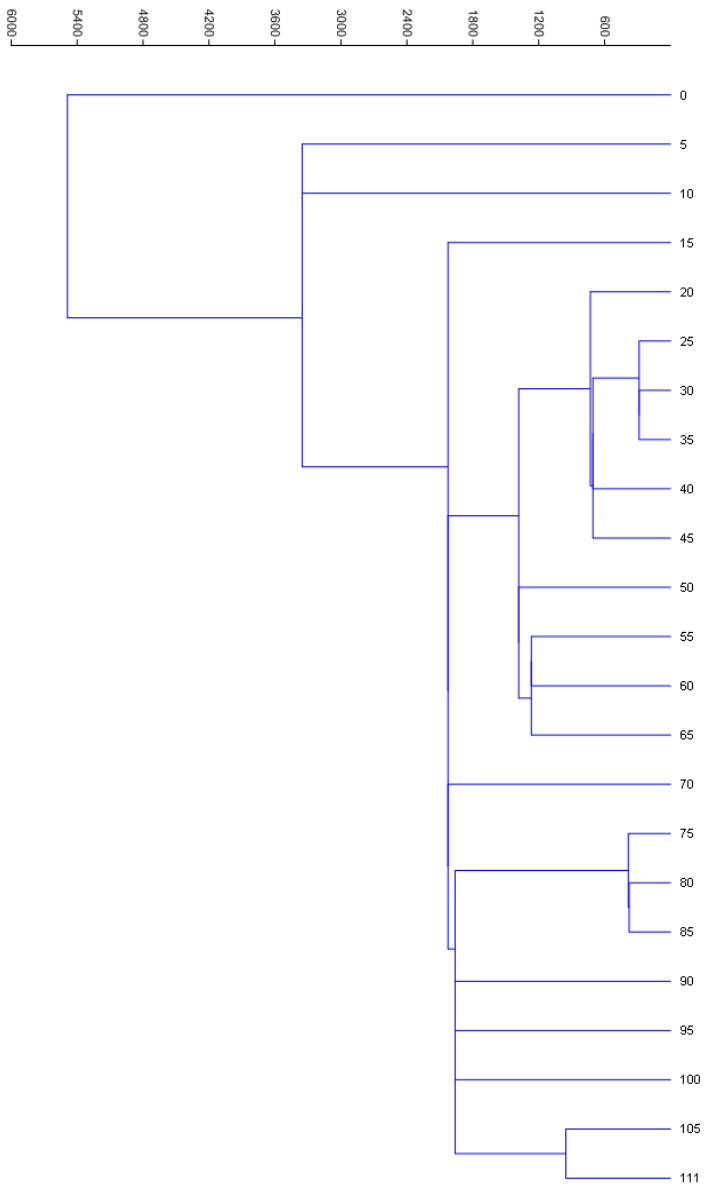
	Chimonodinium spp.		*P. limbatum*		*P. inconspicuum*		*P. gatunense*		*P. volzii*	
Depth (cm)	%A	E	%A	E	%A	E	%A	E	%A	E
0	-	-	-	-	-	-	22.0339	1.0576	3.3898	0.4618
5	17.7419	0.9509	-	-	-	-	61.2903	1.2125	1.6129	0.3136
10	-	-	-	-	-	-	27.2727	1.1770	20.0000	1.0571
15	-	-	-	-	-	-	13.1148	0.8471	6.5574	0.6212
20	-	-	-	-	-	-	8.6957	0.8143	36.9565	1.3949
25	-	-	-	-	-	-	100.0000	0.0000	-	-
30	-	-	-	-	0.1250	0.1621	7.1429	1.3491	28.5714	2.3664
35	-	-	-	-	0.0870	0.1594	28.5714	1.9322	19.0476	1.6795
40	-	-	-	-	-	-	-	-	58.3333	2.7894
45	-	-	-	-	-	-	87.5000	2.2918	-	-
50	-	-	-	-	-	-	17.3913	1.0954	28.2609	1.3012
55	-	-	-	-	0.0385	0.0739	24.0000	1.6742	16.0000	1.4371
60	-	-	-	-	0.0213	0.0413	10.8696	0.8995	45.6522	1.4395
65	-	-	1.8182	0.3531	0.0179	0.0383	70.9091	1.2003	14.5455	0.9318
70	2.2222	0.4307	-	-	0.0217	0.0421	8.8889	0.8315	2.2222	0.4307
75	-	-	-	-	0.0714	0.0808	76.9231	2.2903	-	-
80	-	-	-	-	-	-	7.6923	0.8363	5.3846	1.5646
85	-	-	-	-	0.0250	0.0503	46.1538	1.5646	25.6410	1.3704
90	-	-	-	-	0.0541	0.0729	14.2857	1.1593	17.1429	1.2486
95	-	-	-	-	0.0303	0.0453	65.6250	1.6456	-	-
100	18.1818	1.3364	-	-	-	-	10.9091	0.8239	25.4545	1.1512
105	-	-	1.8519	0.3596	0.0182	0.0370	55.5556	1.3254	9.2593	0.7731
111	-	-	-	-	0.0200	0.0388	8.1633	0.7667	5.1020	1.3997

P. willei		*P. wisconsinense*		Cyst type C/D		Cyst type G		Cyst type J		Cyst type K		Cyst type L	
%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	%A	E
66.1017	1.2079	50.8475	0.5606	-	-	0.0169	0.0329	-	-	-	-	0.0169	0.0329
14.5161	0.8769	4.8387	0.5341	-	-	-	-	-	-	-	-	-	-
40.0000	1.2947	12.7273	0.8808	-	-	-	-	-	-	-	-	-	-
6.5574	0.6212	73.7705	1.1039	-	-	-	-	-	-	-	-	-	-
28.2609	1.3012	17.3913	1.0954	8.6957	0.8143	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
57.1429	2.5923	7.1429	1.3491	-	-	-	-	-	-	-	-	-	-
23.8095	1.8217	28.5714	2.5560	-	-	-	-	-	-	-	-	-	-
25.0000	2.4500	16.6667	2.1086	-	-	-	-	-	-	-	-	-	-
-	-	12.5000	0.9557	-	-	-	-	-	-	-	-	-	-
36.9565	1.3949	10.8696	0.8995	4.3478	0.589333	-	-	-	-	2.1739	0.4214	-	-
4.0000	0.7682	52.0000	1.958431	4.0000	0.76816	-	-	-	-	-	-	-	-
26.0870	1.2690	15.2174	1.0380	2.1739	0.42143	-	-	-	-	-	-	-	-
-	-	9.0909	0.8400	-	-	1.8182	0.3531	1.8182	0.3531	-	-	-	-
6.6667	0.7288	80.0000	1.1687	-	-	-	-	-	-	-	-	-	-
7.6923	1.4485	15.3846	1.1324	-	-	-	-	-	-	-	-	-	-
25.6410	1.3704	10.2564	0.9522	2.5641	0.496079	-	-	-	-	-	-	-	-
17.9487	1.2044	10.2564	1.0051	-	-	-	-	-	-	-	-	-	-
20.0000	1.3252	48.5714	1.6558	-	-	-	-	-	-	-	-	-	-
-	-	34.3750	1.2553	-	-	-	-	-	-	-	-	-	-
27.2727	1.1770	18.1818	1.0193	-	-	-	-	-	-	-	-	-	-
7.4074	0.6985	25.9259	1.2270	-	-	-	-	-	-	-	-	-	-
20.4082	1.1285	18.3673	1.0842	-	-	-	-	-	-	-	-	2.0408	0.3959

Appendix 4d: Dinocyst theca abundance per ml in SP Core 3

Depth (cm)	*Parvodinium* (?) sp.
0	145
5	-
10	420
15	-
20	281
25	-
30	417
35	133
40	103
45	-
50	316
55	40
60	769
65	271
70	620
75	83
80	174
85	155
90	1294
95	241
100	289
105	91
111	217

Appendix 4e: Constrained cluster analysis for SP Core 3



Appendix 5a: Dinocyst raw data for Chem-Core

Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total	SDI
0	19	-	5	1	8	20	8	1	1	1	6	-	51	1.7
7	5	-	-	-	9	5	51	-	-	-	-	-	65	0.7
13	14	-	25	-	6	17	3	1	-	-	1	1	54	1.3
20	19	1	3	-	13	26	16	-	-	-	-	-	59	1.3
25	6	-	13	-	12	24	11	-	-	-	-	-	60	1.3

Appendix 5b: Dinocysts per ml in Chem-Core

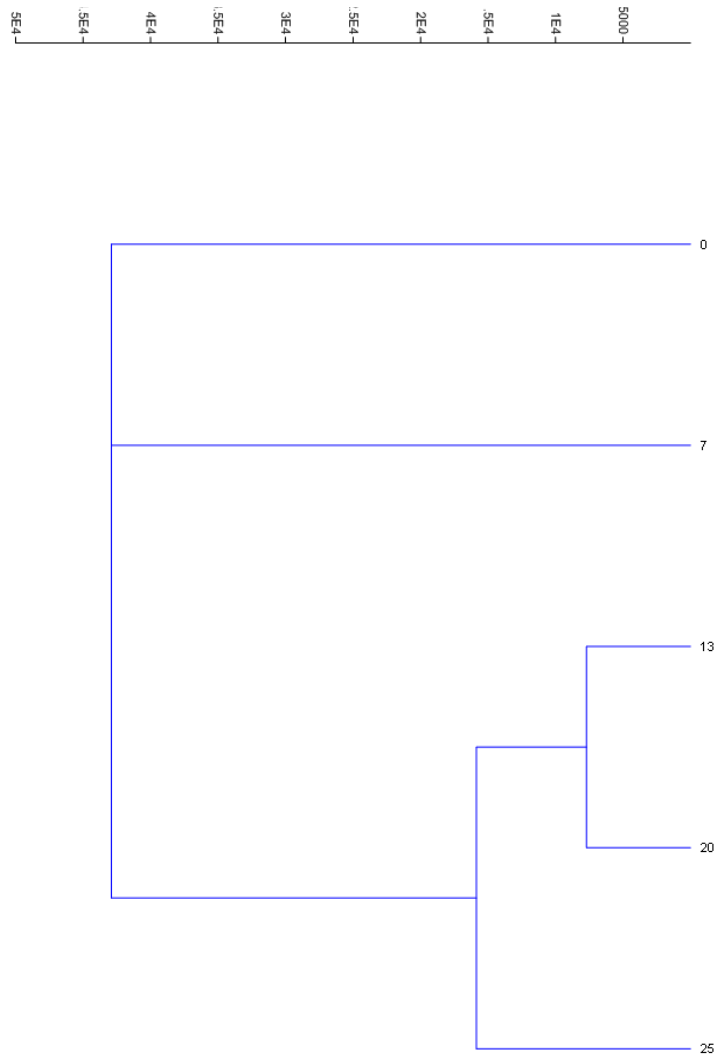
Depth (cm)	Lycopodium	*Chimonodinium* spp.	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total
0	19	-	1142	228	1827	4568	1827	228	228	228	1371	-	11649
7	5	-	-	-	7812	4340	44268	-	-	-	-	-	56420
13	14	-	7750	-	1860	5270	930	310	-	-	310	310	16740
20	19	228	685	-	2969	5939	3655	-	-	-	-	-	13477
25	6	-	9403	-	8680	17360	7957	-	-	-	-	-	43400

Appendix 5c: Dinocyst percent abundance and percent error in Chem-Core

	Chimonodinium spp.		*P. gatunense*		*P. limbatum*		*P. volzii*		*P willei*	
Depth (cm)	%A	E	%A	E	%A	E	%A	E	%A	E
0	-	-	9.8039	0.8161	1.9608	0.3805	15.6863	0.9980	39.2157	1.3400
7	-	-	-	-	-	-	13.8462	0.8398	7.6923	0.6477
13	-	-	46.2963	1.3299	-	-	11.1111	0.8382	31.4815	1.2388
20	1.6949	0.3294	5.0847	0.5603	-	-	22.0339	1.0570	44.0678	1.2668
25	-	-	21.6667	1.0424	-	-	20.0000	1.0121	40.0000	1.2396

P. wisconsinense		Cyst type C/D		Cyst type G		Cyst type J		Cyst type K		Cyst type L		
%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	Total
15.6863	0.9981	1.9608	0.3805	1.9608	0.3805	1.9608	0.3805	11.7647	0.8842	-	-	51
78.4615	0.9994	-	-	-	-	-	-	-	-	-	-	65
5.5556	0.6109	1.8519	0.3594	-	-	-	-	1.8519	0.3594	1.8519	0.3594	54
27.1186	1.1344	-	-	-	-	-	-	-	-	-	-	59
18.3333	0.9791	-	-	-	-	-	-	-	-	-	-	60

Appendix 5d: Constrained cluster analysis for Chem-Core



Appendix 6a: Dinocyst raw data for selected surface sediments

Sample Name	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. limbatum*
SP Core 2	68	1	1	-	-
SP Core 3	30	-	1	13	-
Chem-core	19	-	1	5	1
SG 1	76	1	1	7	-
SG 2	48	18	1	9	-
SG 3	57	5	1	11	-

P. volzii	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total	SDI
1	17	2	-	-	1	-	1	24	1.0
2	39	3	-	1	-	-	1	60	1.1
8	20	8	1	1	1	6	-	52	1.7
9	33	-	-	-	-	-	-	51	0.9
-	27	3	-	-	-	-	-	58	1.2
4	35	4	-	1	-	-	-	61	1.3

Appendix 6b: Dinocysts per ml in selected surface sediments

Sample Name	Lycopodium	*Chimonodinium* spp.	*P. inconspicuum*	*P. gatunense*	*P. limbatum*	*P. volzii*	*P willei*	*P. wisconsinense*	Cyst type C/D	Cyst type G	Cyst type J	Cyst type K	Cyst type L	Total
SP Core 2	68	64	64	-	-	64	1085	128	-	-	64	64	64	1596
SP Core 3	30	-	145	1881	-	289	5642	434	-	145	-	-	145	8680
Chem-core	19	-	228	1142	228	1827	4568	1827	228	228	228	1371	-	11878
SG 1	76	57	57	400	-	514	1884	-	-	-	-	-	-	2912
SG 2	48	1628	90	814	-	-	2441	271	-	-	-	-	-	5244
SG 3	57	417	83	918	-	334	2921	334	-	83	-	-	-	5091

Appendix 6c: Dinocyst percent abundance and percent error in selected surface sediments

	Chimonodinium spp.		*P. inconspicuum*		*P. gatunense*		*P. limbatum*		*P. volzii*	
Sample Name	%A	E	%A	E	%A	E	%A	E	%A	E
SP Core 2	4.1667	0.7995	4.1667	0.7995	-	-	-	-	4.1667	0.7995
SP Core 3	-	-	1.5385	0.3294	21.6667	1.0576	-	-	3.3333	0.4618
Chem-core	-	-	1.9231	0.3733	9.6154	0.8013	1.9231	0.3733	15.3846	0.9807
SG 1	1.9608	0.3805	1.9608	0.3805	13.7255	0.9444	-	-	17.6471	1.0463
SG 2	31.0345	1.1906	1.7241	0.3350	15.5172	0.9318	-	-	-	-
SG 3	8.1967	0.6884	1.6393	0.3187	18.0328	0.9648	-	-	6.5574	0.6212

P. willei		*P. wisconsinense*		Cyst type C/D		Cyst type G		Cyst type J		Cyst type K		Cyst type L		
%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	Raw data total
70.8333	1.8185	8.3333	1.1058	-	-	-	-	4.1667	0.2022	-	-	4.1667	0.7995	24
65.0000	1.2079	5.0000	0.5606	-	-	1.6667	0.3294	-	-	-	-	1.6949	0.3294	60
38.4615	1.3223	15.3846	0.9807	1.9231	0.3733	1.9231	0.3733	1.9231	0.3733	11.5385	0.8684	-	-	52
64.7059	1.3116	-	-	-	-	-	-	-	-	-	-	-	-	51
46.5517	1.2837	5.1724	0.5700	-	-	-	-	-	-	-	-	-	-	58
57.3770	1.2410	6.5574	0.6212	-	-	1.6393	0.3186	-	-	-	-	-	-	61

Appendix 7a: Protozoan raw data for SP09 KC2

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta 'aerophila'*	*C. constricta 'constricta'*	*C. constricta 'spinosa'*	*L. spiralis*	*H. sphagni*	*P. compressa*	*C. tricuspis*	*L. vas*
0	-	-	1	-	-	-	-	-	-	14	-
10	9	77	4	34	37	7	2	-	15	1354	-
20	2	17	-	24	25	2	3	1	9	78	-
30	9	55	-	75	49	7	8	-	4	96	-
40	10	89	-	44	53	9	17	-	4	82	-
50	3	152	7	100	106	14	13	-	16	108	1
60	4	38	2	48	59	19	9	1	8	84	-
70	3	58	1	57	79	28	18	-	10	108	-
90	5	36	3	27	38	4	4	-	8	65	-
100	5	55	7	46	74	10	4	-	43	76	-
110	-	23	2	18	15	11	1	-	28	89	-
140	-	44	-	3	32	-	8	-	43	50	-
170	1	30	1	15	56	-	3	-	19	67	-
191	-	28	-	24	62	-	-	-	54	65	-
221	-	12	-	-	5	1	1	-	4	26	-
251	-	6	2	5	20	-	-	-	1	8	-
291	-	-	-	1	1	-	-	-	-	1	-

D. protaeiformis	*D. bidens*	*D. corona*	*D. fragosa*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total Protozoa	Protozoa/ml	Total theca	SDI
1	-	-	-	-	-	-	-	-	3	162	181	72.4	19	0.8
37	3	3	-	1	-	-	108	16	-	128	1835	734	1707	0.9
11	-	-	-	-	-	-	38	33	-	25	268	268	243	2.0
34	1	-	2	-	-	-	56	37	12	179	624	249.6	445	2.2
31	-	-	-	2	-	-	73	27	4	30	475	190	445	2.2
37	1	1	1	-	-	-	157	35	24	127	903	361.2	776	2.2
36	-	4	-	-	-	-	68	33	18	56	487	194.8	431	2.3
66	-	1	1	-	1	-	106	26	47	358	968	387.2	610	2.3
21	1	-	-	-	-	-	60	44	16	116	448	179.2	332	2.2
24	-	7	1	1	-	-	99	46	28	139	665	266	526	2.3
19	1	1	-	-	-	-	45	8	15	36	312	124.8	276	2.1
20	-	-	-	1	3	-	34	9	10	18	275	110	257	2.1
17	-	-	-	-	2	4	73	4	24	86	402	100.5	316	2.1
9	-	5	1	-	1	-	31	2	4	268	554	138.5	286	2.0
1	-	-	-	1	1	-	1	-	-	4	57	22.8	21.2	1.6
2	-	-	-	-	-	-	17	-	-	1	62	24.8	24.4	1.7
1	-	-	-	-	-	-	4	1	-	-	9	3.6	9	1.6

Appendix 7b: Total protozoans per ml for SP09 KC2

Thecamoebians	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*	*H. sphagni*	*N. collaris*
0	-	-	-	-	-	-	-	-	-
10	4	31	2	14	15	3	1	-	-
20	2	17	-	24	25	2	3	1	-
30	4	22	-	30	20	3	3	-	-
40	4	36	-	18	21	4	7	-	-
50	1	61	3	40	42	6	5	-	-
60	2	15	1	19	24	8	4	-	-
70	1	23	-	23	32	11	7	-	-
90	2	14	1	11	15	2	2	-	-
100	2	22	3	18	30	4	2	-	-
110	-	9	1	7	6	4	-	-	-
140	-	18	-	1	13	-	3	-	-
170	-	8	-	4	14	-	1	-	-
191	-	7	-	6	16	-	-	-	-
221	-	5	-	-	2	-	-	-	-
251	-	2	1	2	8	-	-	-	-
291	-	-	-	-	-	-	-	-	-

P. compressa	*C. tricuspis*	*L. vas*	*D. protaeiformis*	*D. bidens*	*D. corona*	*D. fragosa*	*D. urens*	*D. urceolata*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total theca/ml
-	6	-	-	-	-	-	-	-	-	-	1	65	8
6	542	-	15	1	1	-	-	-	43	6	-	51	683
9	78	-	11	-	-	-	-	-	38	33	-	25	243
2	38	-	14	-	-	1	-	-	22	15	5	72	178
2	33	-	12	-	-	-	1	-	29	11	2	12	178
6	43	-	15	-	-	-	-	-	63	14	10	51	310
3	34	-	14	-	2	-	-	-	27	13	7	22	172
4	43	-	26	-	-	-	-	-	42	10	19	143	244
3	26	-	8	-	-	-	-	-	24	18	6	46	133
17	30	-	10	-	3	-	-	-	40	18	11	56	210
11	36	-	8	-	-	-	-	-	18	3	6	14	110
17	20	-	8	-	-	-	-	1	14	4	4	7	103
5	17	-	4	-	-	-	-	1	18	1	6	22	79
14	16	-	2	-	1	-	-	-	8	1	1	67	72
2	10	-	-	-	-	-	-	-	-	-	-	2	24
-	3	-	1	-	-	-	-	-	7	-	-	-	24
-	-	-	-	-	-	-	-	-	2	-	-	-	4

Appendix 7c: Protozoan percent abundance and percent error in SP09 KC2

	A. vulgaris		*C. aculeata*		*C. discoides*		*C. constricta 'aerophila'*		*C. constricta 'constricta'*		*C. constricta 'spinosa'*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	-	-	-	-	5.2632	1.0041	-	-	-	-	-	-
10	0.5272	0.0344	4.5108	0.0985	0.2343	0.0229	1.9918	0.0663	2.1675	0.0691	0.4101	0.0303
20	0.8230	0.1136	6.9959	0.3207	-	-	9.8765	0.3751	10.2881	0.3820	0.8230	0.1136
30	2.0225	0.1308	12.3596	0.3058	-	-	16.8539	0.3478	11.0112	0.2908	1.5730	0.1156
40	2.2472	0.1377	20.0000	0.3717	-	-	9.8876	0.2773	11.9101	0.3010	2.0225	0.1308
50	0.3866	0.0437	19.5876	0.2792	0.9021	0.0665	12.8866	0.2357	13.6598	0.2416	1.8041	0.0936
60	0.9281	0.0905	8.8167	0.2677	0.4640	0.0642	11.1369	0.2970	13.6891	0.3245	4.4084	0.1938
70	0.4918	0.0555	9.5082	0.9622	0.1639	0.0321	9.3443	0.2310	12.9508	0.2665	4.5902	0.1661
90	1.5291	0.1320	11.0092	0.3367	0.9174	0.1026	8.2569	0.2961	11.6208	0.3447	1.2232	0.1182
100	0.9506	0.0829	10.4563	0.2615	1.3308	0.0979	8.7452	0.2414	14.0684	0.2971	1.9011	0.1167
110	-	-	8.3333	0.3261	0.7246	0.1001	6.5217	0.2913	5.4348	0.2675	3.9855	0.2308
140	-	-	17.1206	0.4605	-	-	1.1673	0.1313	12.4514	0.4037	-	-
170	0.3165	0.0619	9.4937	0.3232	0.3165	0.0619	4.7468	0.2345	17.7215	0.4210	-	-
191	-	-	9.7902	0.3444	-	-	8.3916	0.3213	21.6783	0.4776	-	-
221	-	-	22.6400	1.1267	-	-	-	-	9.4330	0.7868	9.4330	0.7868
251	-	-	9.8361	0.7475	3.2787	0.4456	8.1967	0.6885	32.7869	1.1781	-	-
291	-	-	-	-	-	-	11.1111	2.0531	11.1111	2.0531	-	-

L. spiralis		*H. sphagni*		*P. compressa*		*C. tricuspis*		*L. vas*	
%A	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
-	-	-	-	-	-	73.6842	1.9800	-	-
0.1172	0.0162	-	-	0.8787	0.0443	79.3204	0.1921	-	-
1.2346	0.1388	0.4115	0.0805	3.7037	0.2375	32.0988	0.5870	-	-
1.7978	0.1235	-	-	0.8989	0.0877	21.5730	0.3822	-	-
3.8202	0.1781	-	-	0.8989	0.0877	18.4270	0.3602	-	-
1.6753	0.0903	-	-	2.0619	0.1000	13.9175	0.2435	0.1289	0.0252
2.0882	0.1350	0.2320	0.0454	1.8561	0.1274	19.4896	0.3740	-	-
2.9508	0.1343	-	-	1.6393	0.1008	17.7049	0.3029	-	-
1.2232	0.1182	-	-	2.4465	0.1662	19.8777	0.4293	-	-
0.7605	0.0742	-	-	8.1749	0.2341	14.4487	0.3005	-	-
0.3623	0.0709	-	-	10.1449	0.3562	32.2464	0.5515	-	-
3.1128	0.2123	-	-	16.7315	0.4563	19.4553	0.4840	-	-
0.9494	0.1069	-	-	6.0127	0.2621	21.2025	0.4507	-	-
-	-	-	-	18.8811	0.4536	22.7273	0.4857	-	-
-	-	-	-	7.5472	0.7113	49.0566	1.3459	-	-
-	-	-	-	1.6393	0.3187	13.1148	0.8470	-	-
-	-	-	-	-	-	11.1111	2.0531	-	-

Appendix 7c continued: Protozoan percent abundance and percent error in SP09 KC2

	D. protaeiformis		*D. bidens*		*D. corona*		*D. fragosa*		*D. urens*	
Depth (cm)	%A	E	%A	E	%A	E	%A	E	%A	E
0	5.2632	1.0041	-	-	-	-	-	-	-	-
10	2.1675	0.0691	0.1757	0.0199	0.1757	0.0199	-	-	0.0586	0.0115
20	4.5267	0.2614	-	-	-	-	-	-	-	-
30	7.6404	0.2468	0.2247	0.0440	-	-	0.4494	0.0621	-	-
40	6.9663	0.2365	-	-	-	-	-	-	0.4494	0.0621
50	4.7680	0.1499	0.1289	0.0252	0.1289	0.0252	0.1289	0.0252	-	-
60	8.3527	0.2612	-	-	0.9281	0.0905	-	-	-	-
70	10.8197	0.2465	-	-	0.1639	0.0321	0.1639	0.0321	-	-
90	6.4220	0.2637	0.3058	0.0594	-	-	-	-	-	-
100	4.5627	0.1783	-	-	1.3308	0.0979	0.1901	0.0372	0.1901	0.0372
110	6.8841	0.2987	0.3623	0.0709	0.3623	0.0709	-	-	-	-
140	7.7821	0.3275	-	-	-	-	-	-	0.3891	0.0761
170	5.3797	0.2488	-	-	-	-	-	-	-	-
191	3.1469	0.2023	-	-	1.7483	0.1519	0.3497	0.0684	-	-
221	9.4330	0.7868	-	-	-	-	-	-	0.0943	0.7868
251	3.2787	0.4456	-	-	-	-	-	-	-	-
291	11.1111	2.0531	-	-	-	-	-	-	-	-

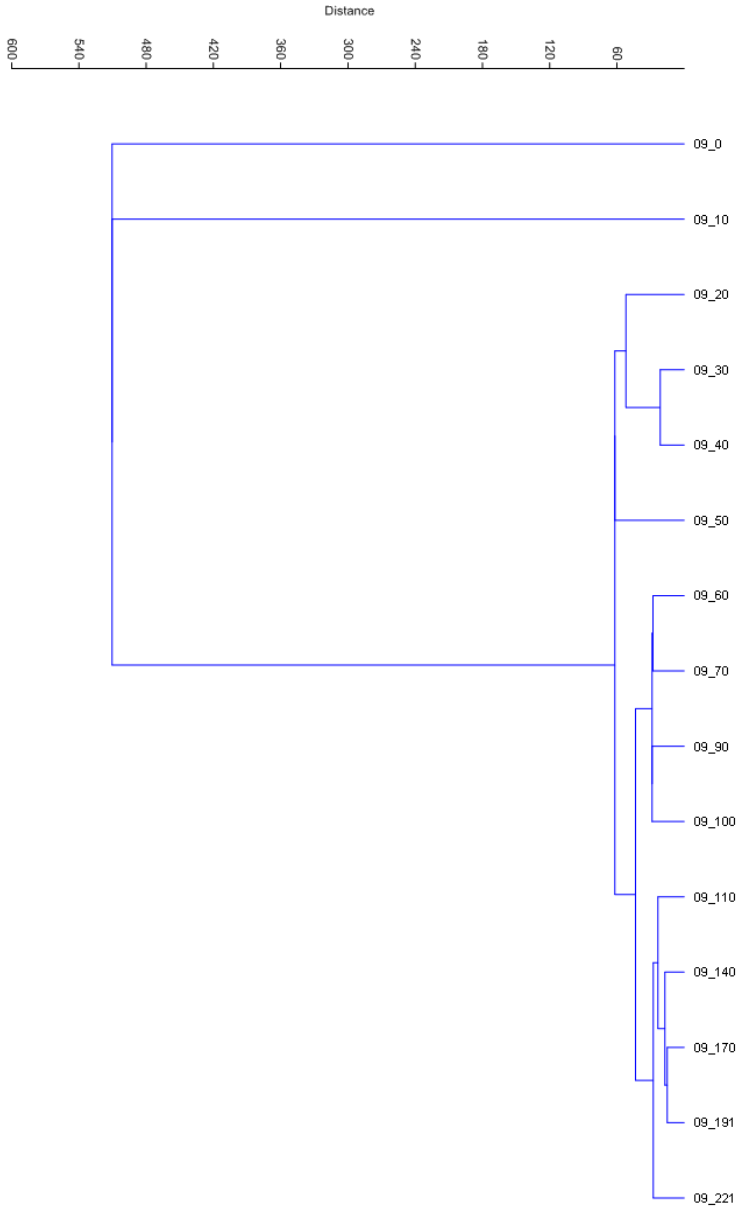
D. urceolata		*D. globula*		*D. oblonga*		*D. bacillifera*		*D. amphora*	
%A	E	%A	E	%A	E	%A	E	%A	E
-	-	-	-	-	-	-	-	15.7895	1.6396
-	-	-	-	6.3269	0.1155	0.9373	0.0457	-	-
-	-	-	-	15.6379	0.4567	13.5802	0.4307	-	-
-	-	-	-	12.5843	0.3082	8.3146	0.2565	2.6966	0.1505
-	-	-	-	16.4045	0.3441	6.0674	0.2218	0.8989	0.0877
-	-	-	-	20.2320	0.2827	4.5103	0.1460	3.0928	0.1218
-	-	-	-	15.7773	0.3442	7.6566	0.2510	4.1763	0.1889
0.1639	0.0321	-	-	17.3770	0.3007	4.2623	0.1603	7.7049	0.2116
-	-	-	-	18.3486	0.4164	13.4557	0.3671	4.8930	0.2320
-	-	-	-	18.8213	0.3340	8.7452	0.2414	5.3232	0.1919
-	-	-	-	16.3043	0.4358	2.8986	0.1979	5.4348	0.2675
1.1673	0.1313	-	-	13.2296	0.4142	3.5019	0.2248	3.8911	0.2364
0.6329	0.0874	1.2658	0.1233	23.1013	0.4647	1.2658	0.1233	7.5949	0.2921
0.3497	0.0684	-	-	10.8392	0.3603	0.6993	0.0966	1.3986	0.1361
-	-	-	-	9.4330	0.7868	-	-	-	-
-	-	-	-	27.8689	1.12517	-	-	-	-
-	-	-	-	44.4444	3.24641	11.1111	2.0531	-	-

Appendix 7d: Age against depth, absolute abundances (concentrations) and relative abundances (percentages) of protozoan microfossils.

		Codonella cratera	Meso-oligotrophic assemblage											
SP09 KC2		lorica/ml	tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%
Depth (cm)	Age	*C. cratera*	*D. oblonga*	*D. oblonga*	*D. urens*	*D. urens*	*D. urceolata*	*D. urceolata*	*C. constricta 'constricta'*	*C. constricta 'constricta'*	*D. corona*	*D. corona*	*P. compressa*	*P. compressa*
0	0.0	64.8	-	-	-	-	-	-	-	-	-	-	-	-
10	159.5	51.2	43.2	6.3 +/- 1.2	0.4	0.6 +/- 0.01	-	-	14.8	2.2 +/- 0.07	1.2	0.2 +/- 0.02	6.0	0.9 +/- 0.04
20	365.0	25.0	38.0	15.3 +/- 0.5	-	-	-	-	25.0	10.2 +/- 0.3.8	-	-	9.0	3.7 +/- 0.2
30	580.6	71.6	22.4	12.6 +/- 0.3	-	-	-	-	19.6	11.0 +/- 0.3	-	-	1.6	0.9 +/- 0.09
40	733.0	12.0	29.2	16.4 +/- 0.3	0.8	0.4 +/- 0.06	-	-	21.2	11.9 +/- 0.3	-	-	1.6	0.9 +/- 0.09
50	1037.4	50.8	62.8	20.2 +/- 0.3	-	-	-	-	42.4	13.6 +/- 0.2	0.4	0.1 +/- 0.03	6.4	2.0 +/- 0.1
60	1276.6	22.4	27.2	15.8 +/- 0.3	-	-	-	-	23.6	13.7 +/- 0.3	1.6	0.9 +/- 0.09	3.2	1.9 +/- 0.1
70	1521.7	143.2	42.4	17.4 +/- 0.3	-	-	0.4	0.2 +/- 0.03	31.6	12.9 +/- 0.3	0.4	0.2 +/- 0.03	4.0	1.6 +/- 0.1
90	2026.3	46.4	24.0	18.3 +/- 0.3	-	-	-	-	15.2	11.6 +/- 0.3	-	-	3.2	2.4 +/- 0.1
100	2284.2	55.6	39.6	18.8 +/- 0.3	0.4	0.2 +/- 0.04	-	-	29.6	14.1 +/- 0.3	2.8	1.3 +/- 0.01	17.2	8.1 +/- 0.2
110	2544.7	14.4	18.0	16.3 +/- 0.4	-	-	-	-	6.0	5.4 +/- 0.3	0.4	0.4 +/- 0.07	11.2	10.1 +/- 0.4
140	3335.8	7.2	13.6	13.2 +/- 0.4	0.4	0.04 +/- 0.08	1.2	1.2 +/- 0.1	12.8	12.4 +/- 0.4	0.0	-	17.2	16.7 +/- 0.5
170	4128.7	21.5	18.3	23.1 +/- 0.4	-	-	0.5	0.6 +/- 0.09	14.0	17.7 +/- 0.7	0.0	-	4.8	6.0 +/- 0.3
191	4678.3	67.0	7.8	10.8 +/- 0.3	-	-	0.3	0.3 +/- 0.07	15.5	21.7 +/- 0.5	1.3	1.7 +/- 0.2	13.5	18.9 +/- 0.5
221	5448.4	1.6	0.4	9.4 +/- 0.7	0.4	0.09 +/- 0.8	-	-	2.0	9.4 +/- 0.8	-	-	1.6	7.5 +/- 0.7
251	6196.3	0.4	6.8	27.9 +/- 1.1	-	-	-	-	8.0	32.8 +/- 1.2	-	-	0.4	1.6 +/- 0.3
291	7156.1	-	1.6	44.4 +/- 3.0	-	-	-	-	0.4	11.1 +/- 2.1	-	-	-	-

		Eutrophic assemblage								Eutrophic (depleted oxygen) assemblage			
SP09 KC2		tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%	tests/ml	%
Depth (cm)	Age	*C. aculeata*	*C. aculeata*	*C. constricta 'aerophila'*	*C. constricta 'aerophila'*	*L. spiralis*	*L. spiralis*	*D. protaeiformis*	*D. protaeiformis*	*A vulgaris*	*A vulgaris*	*C. tricuspis*	*C. tricuspis*
0	0.0	-	4.5 +/- 0.01	-	-	-	-	0.4	5.3 +/- 1.0	-	-	5.6	73. 7 +/- 2.0
10	159.5	30.8	7.0 +/- 0.3	13.6	2.0 +/- 0.07	0.8	0.1 +/- 0.02	14.8	2.2 +/- 0.7	3.6	0.5 +/- 0.03	541.6	79.3 +/- 0.19
20	365.0	17.0	12.4 +/- 0.3	24.0	9.9 +/- 0.4	3.0	1.2 +/- 0.1	11.0	4.5 +/- 0.3	2.0	0.8 +/- 0.1	78.0	32.1 +/- 0.6
30	580.6	22.0	12.4 +/- 0.3	30.0	16.9 +/- 0.3	3.2	1.8 +/- 0.1	13.6	7.6 +/- 0/3	3.6	2.0 +/- 0.1	38.4	21.6 +/- 0.4
40	733.0	35.6	20.0 +/- 0.4	17.6	9.9 +/- 0.3	6.8	3.8 +/- 0.2	12.4	7.0 +/- 0.2	4.0	2.2 +/- 0.1	32.8	18.4 +/- 0.4
50	1037.4	60.8	19.6 +/- 0.3	40.0	13.0 +/- 0.2	5.2	1.7 +/- 0.09	14.8	4.8 +/- 0.2	1.2	0.4 +/- 0.04	43.2	13.9 +/- 0.2
60	1276.6	15.2	8.8 +/- 0.3	19.2	11.1 +/- 0.3	3.6	2.1 +/- 0/1	14.4	8.4 +/- 0.3	1.6	0.9 +/- 0.09	33.6	19.5 +/- 0.4
70	1521.7	23.2	9.5 +/- 1.0	22.8	9.3 +/- 0.2	7.2	3.0 +/- 0.1	26.4	10.8 +/- 0.2	1.2	0.5 +/- 0.06	43.2	17.7 +/- 0.3
90	2026.3	14.4	11.0 +/- 0.3	10.8	8.2 +/- 0.3	1.6	1.2 +/- 0.1	8.4	6.4 +/- 0.3	2.0	1.5 +/- 0.1	26.0	19.9 +/- 0.4
100	2284.2	22.0	10.5 +/- 0.3	18.4	8.7 +/- 0.2	1.6	0.8 +/- 0.0	9.6	4.6 +/- 0.2	2.0	1.0 +/- 0.08	30.4	14.4 +/- 0.3
110	2544.7	9.2	8.3 +/- 0.3	7.2	6.5 +/- 0.3	0.4	0.4 +/- 0.7	7.6	6.9 +/- 0.3	-	-	35.6	32.2 +/- 0.6
140	3335.8	17.6	17.1 +/- 0.5	1.2	1.2 +/- 0.1	3.2	3.1 +/- 0.2	8.0	7.8 +/- 0.3	-	-	20.0	19.5 +/- 0.5
170	4128.7	7.5	9.5 +/- 0.3	3.8	4.7 +/- 0.2	0.8	0.9 +/- 0.1	4.3	5.4 +/- 0.2	0.3	0.3 +/- 0.06	16.8	21.2 +/- 0.5
191	4678.3	7.0	9.8 +/- 0.3	6.0	8.4 +/- 0.3	-	-	2.3	3.1 +/- 0.2	-	-	16.3	22.7 +/- 0.5
221	5448.4	4.8	22.6 +/- 1.1	-	-	0.4	0.4 +/- 0.7	0.4	9.4 +/- 0.8	-	-	10.4	49.1 +/- 0.1
251	6196.3	2.4	9.8 +/- 0.7	2.0	8.2 +/- 0.7	-		0.8	3.3 +/- 0.5	-	-	3.2	13.2 +/- 0.8
291	7156.1	-	-	0.4	11.1 +/- 2.1	-		0.4	11.1 +/- 0.2	-	-	0.4	11.1 +/- 2.0

Appendix 7e: Constrained cluster analysis for SP09 KC2



Appendix 8a: Protozoan raw data for SP07 PC4

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta 'aerophila'*	*C. constricta 'constricta'*	*C. constricta 'spinosa'*	*L. spiralis*	*H. sphagni*	*P. compressa*	*C. tricuspis*	*L. vas*
0	1	6	-	1	11	-	1	-	-	42	-
10	7	146	4	17	3	11	17	1	34	347	4
20	-	285	16	21	14	7	10	1	42	1657	3
40	9	265	2	22	6	12	6	-	33	122	1
50	11	203	3	48	-	75	2	-	11	96	2
70	21	105	-	22	30	39	-	1	10	71	1
80	16	162	6	46	71	32	3	1	10	99	-
90	5	79	-	17	15	21	-	-	-	22	-
100	-	3	-	-	-	-	-	-	-	-	-
130	-	5	-	-	1	-	-	-	-	1	-

D. protaeiformis	*D. bidens*	*D. corona*	*D. urens*	*D. urceolata*	*D. oblonga*	*D. bacillifera*	*C. cratera*	Total Protozoa	Protozoa/ml	Total theca	SDI
12	-	-	-	-	12	-	88	174	34.8	86	1.5
14	1	30	7	1	80	42	27	793	317.2	766	1.8
39	0	31	1	1	87	35	37	2287	914.8	2250	1.1
25	1	10	2	3	111	12	15	657	262.8	642	1.8
42	1	1	1	-	101	22	41	660	660	619	1.9
27	-	1	-	-	39	10	2	379	379	377	2.1
10	-	-	2	2	60	9	10	539	215.6	529	2.0
1	-	1	3	-	102	2	1	269	107.6	268	1.7
-	-	-	-	-	4	-	-	-	2.8	7	0.7
-	-	-	1	-	2	-	-	-	4	10	1.4

Appendix 8b: Total protozoans per ml for SP07 KC2

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*
0	0	1	-	0	2	-	0
10	3	58	2	7	1	4	7
20	-	114	6	8	6	3	4
40	4	106	1	9	2	5	2
50	11	203	3	48	-	75	2
70	15	309	4	57	2	80	4
80	6	65	2	18	28	13	1
90	2	32	-	7	6	8	-
100	-	1	-	-	-	-	-
130	-	2	-	-	0	-	-

P. compressa	*C. tricuspis*	*L. vas*	*D. protaeiformis*	*D. bidens*	*D. corona*	*D. urens*	*D. urceolata*	*D. oblonga*	*D. bacillifera*	*C. cratera*	Total theca/ml
-	8	-	2	-	-	-	-	2	-	18	17
14	139	2	6	0	12	3	0	32	17	11	306
17	663	1	16	-	12	0	0	35	14	15	900
13	49	0	10	0	4	1	1	44	5	6	257
11	96	2	42	1	1	1	0	101	22	41	619
24	145	2	52	1	5	2	1	145	27	47	876
4	40	-	4	-	-	1	1	24	4	4	212
-	9	-	0	-	0	1	-	41	1	0	107
-	-	-	-	-	-	-	-	2	-	-	3
-	0	-	-	-	-	0	-	1	-	-	4

Appendix 8c: Protozoan percent abundance and percent error in SP07 PC4

	A. vulgaris		*C. aculeata*		*C. discoides*		*C. constricta 'aerophila'*		*C. constricta 'constricta'*		*C. constricta 'spinosa'*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	1.2658	0.2363	7.5949	0.5599	-	-	1.2658	0.2363	13.9241	0.7317	-	-
10	0.9138	0.0674	19.0601	0.2782	0.5222	0.0510	2.2193	0.1043	0.3916	0.0442	1.4360	0.0843
20	-	-	12.6667	0.1374	0.7111	0.0347	0.9333	0.0397	0.6222	0.0325	0.3111	0.0230
40	1.4019	0.0909	41.2773	0.3808	0.3115	0.0431	3.4268	0.1407	0.9346	0.0744	1.8692	0.1048
50	1.7771	0.1041	32.7948	0.3698	0.4847	0.0547	7.7544	0.2107	-	-	1.8692	0.1048
70	5.5703	0.2315	27.8515	0.4525	-	-	5.8355	0.2366	-	-	10.3448	0.3074
80	3.0246	0.1459	30.6238	0.3928	1.1342	0.0902	8.6957	0.2401	13.4216	0.2905	6.0491	0.2032
90	1.8657	0.1620	29.4776	0.5459	-	-	6.3433	0.2918	5.5970	0.2752	7.8358	0.3217
100	-	-	42.8600	3.65633	-	-	-	-	-	-	-	-
130	-	-	50.0000	0.3099	-	-	-	-	10.0000	1.8590	-	-

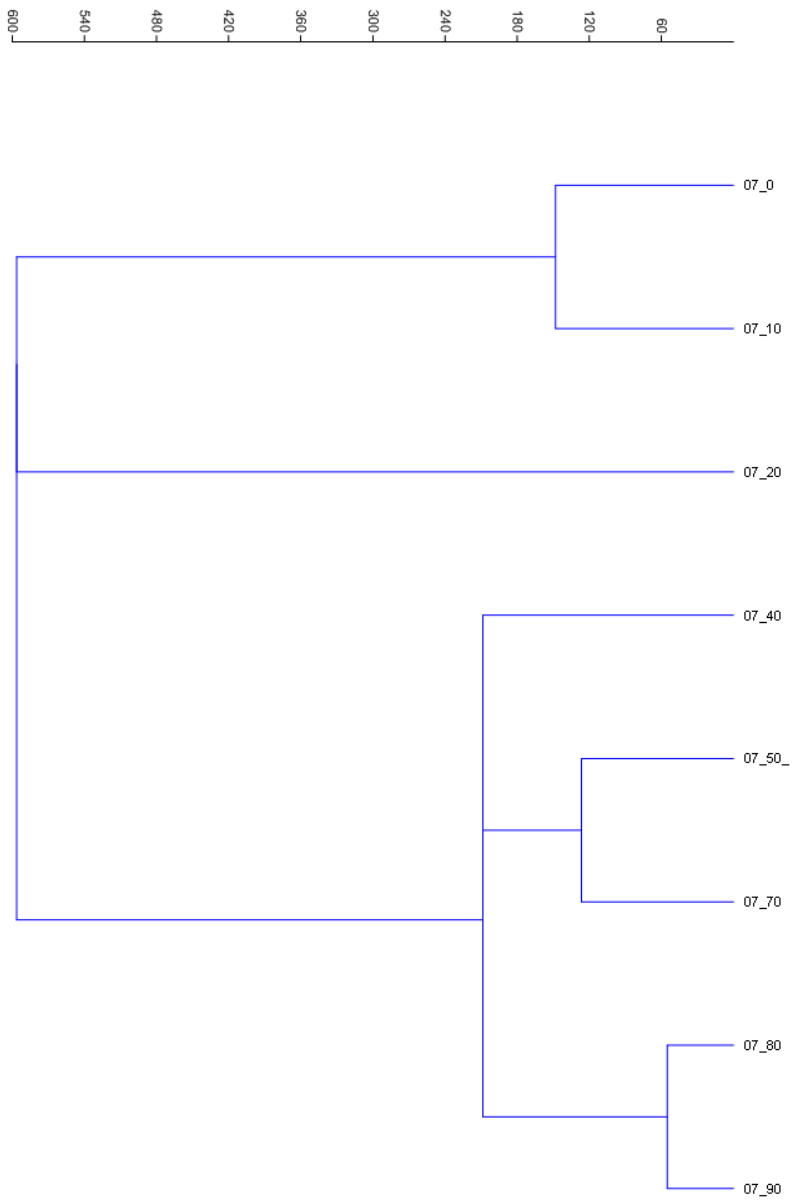
L. spiralis		*H. sphagni*		*P. compressa*	
%A	**E**	**%A**	**E**	**%A**	**E**
1.2658	0.2363	-	-	-	-
2.2193	0.1043	0.1305	0.0256	4.4386	0.1459
0.4444	0.0275	0.0444	0.0087	1.8667	0.0559
0.9346	0.0744	-	-	5.1402	0.1708
0.3231	0.0447	-	-	1.7771	0.1041
-	-	0.2653	0.0519	2.6525	0.1622
0.5671	0.0640	0.1890	0.0370	1.8904	0.1161
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-

Appendix 8c continued: Protozoan percent abundance and percent error in SP07 PC4

	C. tricuspis		*L. vas*		*D. protaeiformis*		*D. bidens*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	53.1646	1.0546	-	-	15.1899	0.7586	-	-
10	45.3003	0.3525	0.5222	0.0510	1.8277	0.0949	0.1305	0.0256
20	73.6444	0.1820	0.1333	0.0151	1.7333	0.0539	-	-
40	19.0031	0.3035	0.1558	0.0305	3.8941	0.1496	0.1558	0.0305
50	15.5089	0.2852	0.3231	0.0447	6.7851	0.1981	0.1616	0.0316
70	18.8329	0.3947	0.2653	0.0519	7.1618	0.2603	-	-
80	18.7146	0.3324	-	-	1.8904	0.1161	-	-
90	8.2090	0.3286	-	-	0.3731	0.0730	-	-
100	-	-	-	-	-	-	-	-
130	10.0000	1.8590	-	-	-	-	-	-

D. corona		*D. urens*		*D. urceolata*		*D. oblonga*		*D. bacillifera*	
%A	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
-	-	-	-	-	-	15.1899	0.7586	-	-
3.9164	0.1374	0.9138	0.0674	0.1305	0.0256	10.4439	0.2166	5.4830	0.1612
1.3778	0.0482	0.0444	0.0087	0.0444	0.0087	3.8667	0.0797	1.5556	0.0511
1.5576	0.0958	0.3115	0.0431	0.4673	0.0528	17.2897	0.2925	1.8692	0.1048
0.1616	0.0316	0.1616	0.0316	-	-	16.3166	0.2911	3.5541	0.1459
0.2653	0.0519	-	-	-	-	10.3448	0.3074	2.6525	0.1622
-	-	0.3781	0.0523	0.3781	0.0523	11.3422	0.2702	1.7013	0.1102
0.3731	0.0730	1.1194	0.1260	-	-	38.0597	0.5813	0.7463	0.1030
-	-	-	-	-	-	57.14	3.66609	-	-
-	-	10.0000	1.8590	-	-	20.0000	2.4790	-	-

Appendix 8d: Constrained cluster analysis for SP07 PC4



Appendix 9a: Protozoan raw data for SP Core 2

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*	*P. compressa*	*C. tricuspis*
0	2	21	1	13	51	-	3	40	48
8	1	22	1	3	34	3	-	34	6
10	-	15	-	1	4	-	-	3	-
15	-	19	5	26	19	-	-	-	1
20	-	3	2	-	-	-	-	-	-
25	1	15	-	2	6	6	-	-	1
30	-	33	-	-	12	-	-	-	1
35	-	31	1	2	7	-	-	2	-
40	3	122	2	4	116	40	-	-	4
45	-	26	-	-	3	-	-	-	-
50	1	75	-	-	13	-	-	-	-
55	-	44	-	-	3	3	-	-	-
60	-	1	-	3	7	1	-	-	-
70	-	67	-	-	3	7	-	-	-
74	2	152	-	8	45	23	-	-	2

protaeiform	*D. bidens*	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*C. cratera*	Total Protozoans	Protozoans/ml	SDI
18	3	7	-	10	-	158	-	7	382	109.1428571	1.9
6	4	17	2	9	68	101	-	-	311	62.2	2.0
-	-	-	-	-	-	28	-	-	51	10.2	1.1
-	-	-	-	-	-	16	1	-	87	17.4	1.6
-	-	-	6	-	-	44	-	-	55	11	0.7
-	-	-	1	1	-	34	-	-	67	13.4	1.5
-	-	-	-	-	-	6	-	-	52	20.8	1.0
-	-	-	2	1	-	16	-	-	62	12.4	1.4
1	1	1	9	1	-	41	-	-	345	69	1.6
-	-	-	12	-	-	11	-	-	52	10.4	1.2
-	-	-	2	-	-	16	-	-	107	21.4	0.9
-	-	-	-	-	-	7	-	-	57	11.4	0.1
-	-	-	1	-	-	1	-	-	14	2.8	1.4
2	-	-	-	-	-	47	-	-	126	25.2	1.0
-	-	-	2	-	-	11	1	-	246	98.4	1.2

Appendix 9b: Total protozoans per ml for SP Core 2

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*
0	1	6	-	4	15	-	1
8	-	4	-	1	7	1	-
10	-	3	-	-	1	-	-
15	-	4	1	5	4	-	-
20	-	1	-	-	-	-	-
25	-	3	-	-	1	1	-
30	-	13	-	-	5	-	-
35	-	6	-	-	1	-	-
40	1	24	-	1	23	8	-
45	-	5	-	-	1	-	-
50	-	15	-	-	3	-	-
55	-	9	-	-	1	1	-
60	-	-	-	1	1	-	-
70	-	13	-	-	1	1	-
74	1	61	-	3	18	9	-

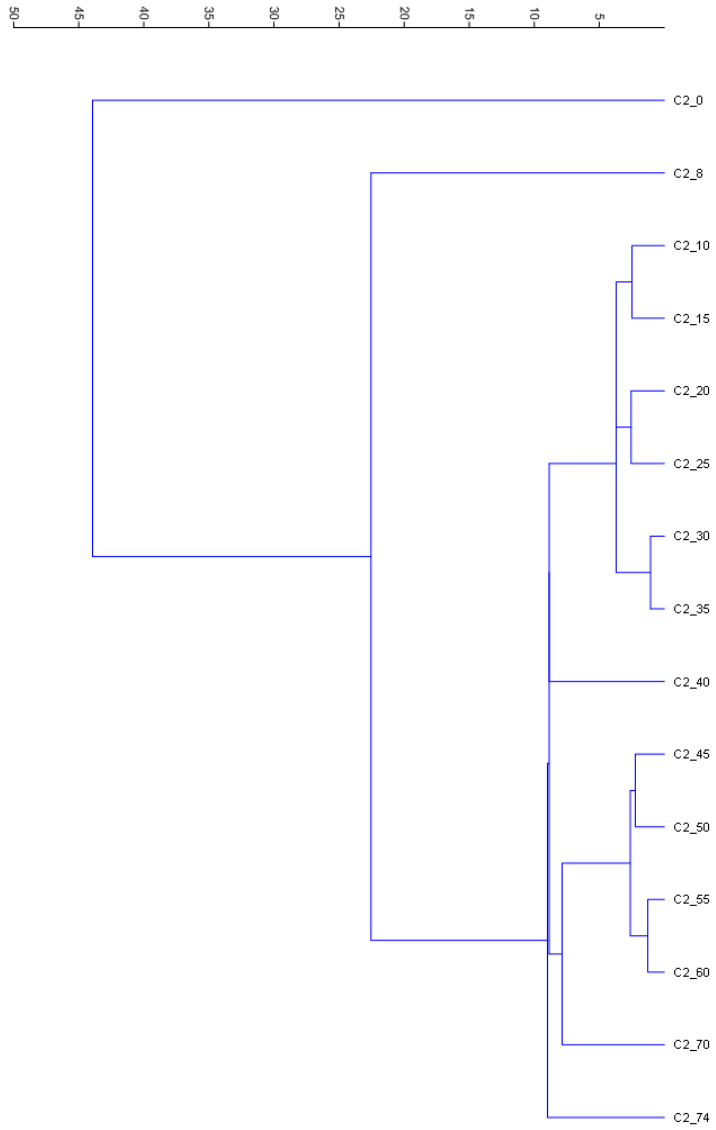
P. compressa	*C. tricuspis*	*D. protaeiformis*	*D. bidens*	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*C. cratera*
11	14	5	1	2	-	3	-	45	-	2
7	1	1	1	3	-	2	14	20	-	-
1	-	-	-	-	-	-	-	6	-	-
-	-	-	-	-	-	-	-	3	-	-
-	-	-	-	-	1	-	-	9	-	-
-	-	-	-	-	-	-	-	7	-	-
-	-	-	-	-	-	-	-	2	-	-
-	-	-	-	-	-	-	-	3	-	-
-	1	-	-	-	2	-	-	8	-	-
-	-	-	-	-	2	-	-	2	-	-
-	-	-	-	-	-	-	-	3	-	-
-	-	-	-	-	-	-	-	1	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	9	-	-
-	1	-	-	-	1	-	-	4	-	-

Appendix 9c: Protozoan percent abundance and percent error in SP Core 2

	A. vulgaris		*C. aculeata*		*C. discoides*		*C. constricta aerophila*		*C. constricta constricta*		*C. constricta spinosa*		*L. spiralis*		*P. compressa*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	0.5333	0.0737	5.6000	0.2327	0.2667	0.0522	3.4667	0.1852	13.6000	0.3469	-	-	0.8000	0.0902	10.6667	0.3124
8	0.3215	0.0629	7.0740	0.2850	0.3215	0.0629	0.9646	0.1086	10.9325	0.3468	0.9646	0.1086	-	-	10.9325	0.3468
10	-	-	29.4118	1.2505	-	-	1.9608	0.3805	7.8431	0.7379	-	-	-	-	5.8824	0.6458
15	-	-	21.8391	0.8682	5.7471	0.4891	0.2989	0.9621	0.2184	0.8682	-	-	-	-	-	-
20	-	-	5.4545	0.6002	3.6364	0.4947	-	-	-	-	-	-	-	-	-	-
25	1.4925	0.2903	22.3881	0.9981	-	-	2.9851	0.4075	8.9552	0.6837	8.9552	0.6837	-	-	-	-
30	-	-	63.4615	1.3088	-	-	-	-	23.0769	1.1452	-	-	-	-	-	-
35	-	-	50.0000	1.2446	1.6129	0.3136	3.2258	0.4398	11.2903	0.7878	-	-	-	-	3.2258	0.4398
40	0.8696	0.0980	35.3623	0.5045	0.5797	0.0801	1.1594	0.1130	33.6232	0.4985	11.5942	0.3378	-	-	-	-
45	-		50.0000	1.3590	-	-	-	-	5.5556	0.6226	-	-	-	-	-	-
50	0.9346	0.1823	70.0935	0.8675	-	-	-	-	12.1495	0.6190	-	-	-	-	-	-
55	-	-	77.1930	1.0893	-	-	-	-	5.2632	0.5797	5.2632	0.5797	-	-	-	-
60	-	-	7.1429	1.3491	-	-	21.4286	2.1494	50.0000	2.6192	7.1429	1.3491	-	-	-	-
70	-	-	53.1746	0.8713	-	-	-	-	2.3810	0.2662	5.5556	0.4000	-	-	-	-
74	0.8130	0.1122	61.7886	0.6072	-	-	3.2520	0.2217	18.2927	0.4831	9.3496	0.3638	-	-	-	-

C. tricuspis		*D. protaeiformis*		*D. bidens*		*D. corona*		*D. urens*		*D. urceolata*		*D. globula*		*D. oblonga*		*D. bacillifera*	
%A	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
12.8000	0.3381	4.8000	0.2164	0.8000	0.0902	1.8667	0.1370	-	-	2.6667	0.1631	-	-	42.1333	0.4998	-	-
1.9293	0.1529	1.9293	0.1529	1.2862	0.1252	5.4662	0.2526	0.6431	0.0888	2.8939	0.1863	21.8650	0.4594	32.4759	0.5205	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	54.9020	1.3657	-	-
1.1494	0.2240	-	-	-	-	-	-	-	-	-	-	-	-	18.3908	0.8141	0.0115	0.2240
-	-	-	-	-	-	-	-	10.9091	0.8239	-	-	-	-	80.0000	1.0571	-	-
1.4925	0.2903	-	-	-	-	-	-	1.4925	0.2903	1.4925	0.2903	-	-	50.7463	1.1971	-	-
1.9231	0.3733	-	-	-	-	-	-	-	-	-	-	-	-	11.5385	0.8684	-	-
-	-	-	-	-	-	-	-	3.2258	0.4398	1.6129	0.3136	-	-	25.8065	1.0892	-	-
1.1594	0.1130	0.2899	0.0567	0.2899	0.0567	0.2899	0.0567	2.6087	0.1682	0.2899	0.0567	-	-	11.8841	0.3415	-	-
-	-	-	-	-	-	-	-	23.0769	1.1452	-	-	-	-	20.3704	1.0947	-	-
-	-	-	-	-	-	-	-	1.8692	0.2566	-	-	-	-	14.9533	0.6757	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.2807	0.8521	-	-
-	-	-	-	-	-	-	-	7.1429	1.3491	-	-	-	-	7.1429	1.3491	-	-
-	-	1.5873	0.2182	-	-	-	-	-	-	-	-	-	-	37.3016	0.8444	-	-
0.8130	0.1122	-	-	-	-	-	-	0.8130	0.1122	-	-	-	-	4.4715	0.2583	0.4065	0.0795

Appendix 9d: Constrained cluster analysis for SP Core 2



Appendix 10a: Protozoan raw data for SP Core 3

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*	*P. compressa*
0	-	4	-	5	19	-	1	18
5	-	31	2	4	52	-	6	8
10	1	8	13	5	53	-	1	73
15	-	32	13	1	66	-	-	21
20	-	5	-	2	17	-	-	-
25	-	38	-	4	12	-	-	-
30	-	36	-	5	10	-	-	-
35	-	60	-	-	14	18	-	-
40	1	70	-	2	49	4	-	-
45	-	7	-	-	-	-	-	-
50	1	70	2	-	36	-	-	-
55	-	42	-	1	6	3	-	-
60	5	112	-	12	71	29	1	-
65	-	80	-	-	6	-	-	-
70	2	59	-	1	15	5	-	-
75	-	142	-	-	16	11	-	-
80	1	124	-	7	68	12	-	1
85	-	112	2	-	22	14	-	-
90	1	99	1	1	126	17	-	-
95	-	73	-	-	24	14	-	-
100	-	119	-	1	-	32	-	-
105	-	219	-	-	28	-	-	-
111	3	63	1	23	87	15	-	-

C. tricuspis	*protaeiform*	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*C. cratera*	ɔtal Protozoa	Protozoans/ml	SDI
12	3	4	1	8	-	242	-	6	323	129.2	1.0
84	8	4	1	0	-	406	-	5	611	122.2	1.2
90	18	17	8	11	-	445	-	4	747	149.4	1.4
52	5	56	18	23	-	337	-	1	628	251.2	1.6
2	2	3	9	2	-	75	-	-	117	23.4	1.3
-	-	-	-	-	-	17	-	-	71	14.2	1.1
2	-	-	-	-	-	11	-	-	64	12.8	1.2
-	-	-	2	-	-	18	-	-	112	22.4	1.3
-	-	-	3	-	-	11	-	-	140	28	1.2
-	-	-	1	-	-	13	-	-	21	8.4	1.3
-	-	-	6	2	-	56	-	-	173	34.6	1.2
-	-	-	7	-	-	13	-	-	72	14.4	1.2
-	-	-	2	1	-	10	-	-	243	48.6	1.4
-	-	-	5	-	-	6	-	-	97	19.4	0.7
-	-	-	6	-	-	2	-	-	90	36	1.1
1	-	-	7	-	-	6	-	-	183	36.6	0.8
-	4	-	2	-	-	26	1	-	246	24.6	1.4
-	-	-	6	-	11	-	-	-	167	33.4	1.1
6	5	-	16	3	-	29	-	-	304	60.8	1.5
-	-	-	15	4	-	4	-	-	134	26.8	1.3
-	-	-	-	1	-	19	-	-	172	34.4	0.9
-	-	-	8	-	-	28	-	-	283	56.6	0.8
2	5	-	15	2	-	31	-	-	247	49.4	1.8

Appendix 10b: Total protozoans per ml for SP Core 3

Depth (cm)	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*
0	-	2	-	2	8	-	-
5	-	6	-	1	10	-	1
10	-	2	3	1	11	-	-
15	-	13	5	-	26	-	-
20	-	1	-	-	3	-	-
25	-	8	-	1	2	-	-
30	-	7	-	1	2	-	-
35	-	12	-	-	3	4	-
40	-	14	-	-	10	1	-
45	-	3	-	-	-	-	-
55	-	8	-	-	1	1	-
60	1	22	-	2	14	6	-
65	-	16	-	-	1	-	-
70	1	24	-	-	6	2	-
75	-	28	-	-	3	2	-
80	-	12	-	1	7	1	-
85	-	22	-	-	4	3	-
90	-	20	-	-	25	3	-
95	-	15	-	-	5	3	-
100	-	24	-	-	-	6	-
105	-	44	-	-	6	-	-
111	1	13	-	5	17	3	-

P. compressa	*C. tricuspis*	*L. vas*	*rotaeifor*	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*C. cratera*	Total
7	5	-	1	2	-	3	-	97	-	2	129
2	17	-	2	1	-	-	-	81	-	1	122
15	18	-	4	3	2	2	-	89	-	1	149
8	21	1	2	22	7	9	-	135	-	-	251
-	-	-	-	1	2	-	-	15	-	-	23
-	-	-	-	-	-	-	-	3	-	-	14
-	-	-	-	-	-	-	-	2	-	-	13
-	-	-	-	-	-	-	-	4	-	-	22
-	-	-	-	-	1	-	-	2	-	-	25
-	-	-	-	-	-	-	-	5	-	-	8
-	-	-	-	-	1	-	-	3	-	-	14
-	-	-	-	-	-	-	-	2	-	-	49
-	-	-	-	-	1	-	-	1	-	-	19
-	-	-	-	-	2	-	-	1	-	-	36
-	-	-	-	-	1	-	-	1	-	-	37
-	-	-	-	-	-	-	-	3	-	-	25
-	-	-	-	-	1	-	2	-	-	-	33
-	1	-	1	-	3	3	-	6	-	-	63
-	-	-	-	-	3	4	-	1	-	-	30
-	-	-	-	-	-	-	-	4	-	-	34
-	-	-	-	-	2	-	-	6	-	-	57
-	-	-	1	-	3	-	-	6	-	-	49

Appendix 10c: Protozoan percent abundance and percent error in SP Core 3

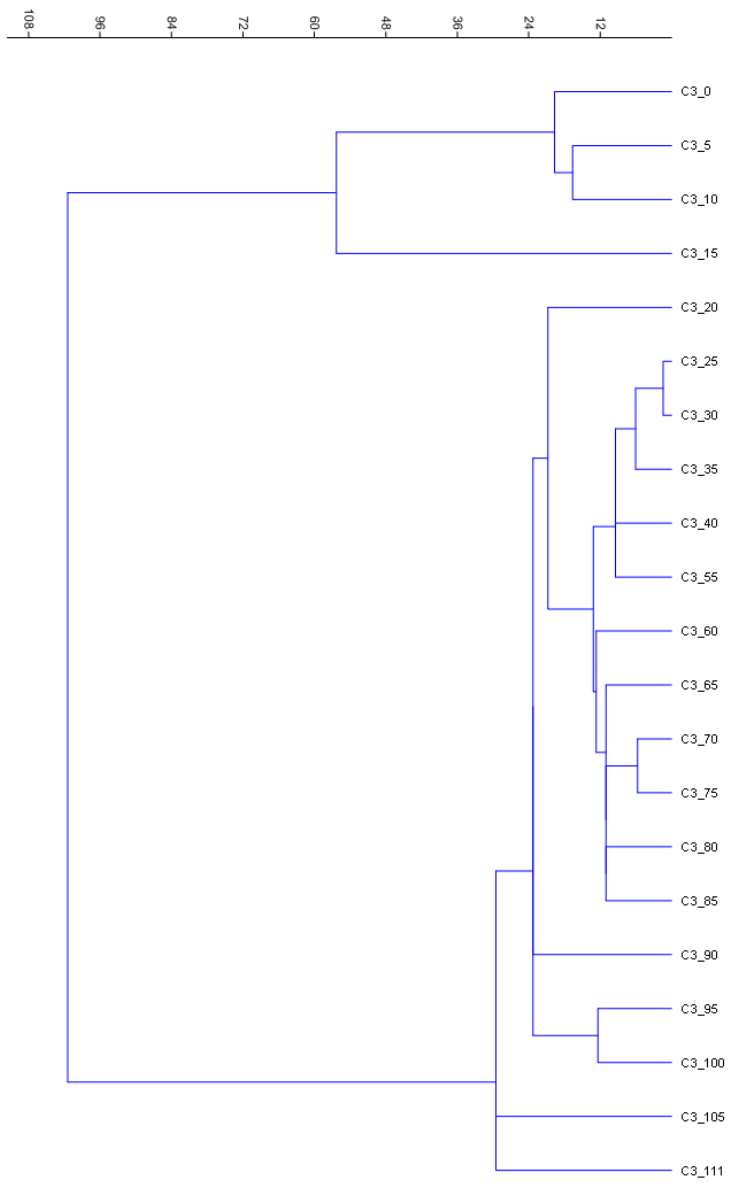
	A. vulgaris		*C. aculeata*		*C. discoides*		*C. constricta aerophila*		*C. constricta constricta*	
Depth (cm)	%A	E	%A	E	%A	E	%A	E	%A	E
0	-	-	1.2618	0.1229	-	-	1.5773	0.1372	5.9937	0.2613
5	-	-	5.1155	0.1754	0.3300	0.0457	0.6601	0.0645	8.5809	0.2230
10	0.1346	0.0264	1.0767	0.0742	1.7497	0.0943	0.6729	0.0588	7.1332	0.1851
15	-	-	5.1037	0.1723	2.0734	0.1115	0.1595	0.0312	10.5263	0.2402
20	-	-	4.2735	0.3665	-	-	1.7094	0.2349	14.5299	0.6386
25	-	-	53.5211	1.1602	-	-	5.6338	0.5363	16.9014	0.8717
30	-	-	56.2500	1.2154	-	-	7.8125	0.6575	15.6250	0.8896
35	-	-	53.5714	0.9236	-	-	-	-	12.5000	0.6125
40	0.7143	0.1395	50.0000	0.8283	-	-	1.4286	0.1966	35.0000	0.7901
45	-	-	33.3333	2.0162	-	-	-	-	-	-
50	-	-	58.3333	1.1388	-	-	1.3889	0.2703	8.3333	0.6384
55	-	-	58.3333	1.1388	-	-	1.3889	0.2703	8.3333	0.6384
60	2.0576	0.1785	46.0905	0.6267	-	-	4.9383	0.2724	29.2181	0.5718
65	-	-	82.4742	0.7566	-	-	-	-	6.1856	0.4794
70	2.2222	0.3045	65.5556	0.9817	-	-	1.1111	0.2166	16.6667	0.7700
75	-	-	77.5956	0.6041	-	-	-	-	8.7432	0.4093
80	0.4065	0.0795	50.4065	0.6248	-	-	2.8455	0.2078	27.6423	0.5589
85	-	-	67.0659	0.7128	1.1976	0.1650	-	-	13.1737	0.5130
90	0.3289	0.0644	32.5658	0.5268	0.3289	0.0644	0.3289	0.0644	41.4474	0.5538
95	-	-	54.4776	0.8432	-	-	-	-	17.9104	0.6492
100	-	-	69.1860	0.6900	-	-	0.5814	0.1136	-	-
105	-	-	77.3852	0.4874	-	-	-	-	9.8940	0.3479
111	1.2146	0.1366	25.5061	0.5436	0.4049	0.0792	9.3117	0.3624	35.2227	0.5957

C. constricta spinosa		*L. spiralis*		*P. compressa*	
%A	E	%A	E	%A	E
-	-	0.3155	0.0617	5.6782	0.2548
-	-	0.9901	0.0788	1.3201	0.0909
-	-	0.1346	0.0264	9.8250	0.2140
-	-	-	-	3.3493	0.1408
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
16.0714	0.6802	-	-	-	-
2.8571	0.2760	-	-	-	-
-	-	-	-	-	-
4.1667	0.4616	-	-	-	-
4.1667	0.4616	-	-	-	-
11.9342	0.4076	0.4115	0.0805	-	-
-	-	-	-	-	-
5.5556	0.4732	-	-	-	-
6.0109	0.3444	-	-	-	-
4.8780	0.2692	-	-	0.4065	0.0795
8.3832	0.4203	-	-	-	-
5.5921	0.2583	-	-	-	-
10.4478	0.5179	-	-	-	-
18.6047	0.5816	-	-	-	-
-	-	-	-	-	-
6.0729	0.2979	-	-	-	-

Appendix 10c continued: Protozoan percent abundance and percent error in SP Core 3

C. tricuspis		*L. vas*		*D. protaeiformis*		*D. corona*		*D. urens*		*D. urceolata*		*D. globula*		*D. oblonga*		*D. bacillifera*	
%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	%A	E	%A	E
3.7855	0.2101	-	-	0.9464	0.1066	1.2618	0.1229	0.3155	0.0617	2.5237	0.1727	-	-	76.3407	0.4678	-	-
13.8614	0.2751	-	-	1.3201	0.0909	0.6601	0.0645	0.1650	0.0323	-	-	-	-	66.9967	0.3744	-	-
12.1131	0.2346	-	-	2.4226	0.1106	2.2880	0.1075	1.0767	0.0742	1.4805	0.0868	-	-	59.8923	0.3524	-	-
8.2935	0.2159	0.4785	0.0540	0.7974	0.0696	8.9314	0.2232	2.8708	0.1307	3.6683	0.1471	-	-	53.7480	0.3903	-	-
1.7094	0.2349	-	-	1.7094	0.2349	2.5641	0.2864	7.6923	0.4828	1.7094	0.2349	-	-	64.1026	0.8692	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.9437	0.9926	-	-
3.1250	0.4263	-	-	-	-	-	-	-	-	-	-	-	-	17.1875	0.9243	-	-
-	-	-	-	-	-	-	-	1.7857	0.2453	-	-	-	-	16.0714	0.6802	-	-
-	-	-	-	-	-	-	-	2.1429	0.2399	-	-	-	-	7.8571	0.4457	-	-
-	-	-	-	-	-	-	-	4.7619	0.9108	-	-	-	-	61.9048	2.0771	-	-
-	-	-	-	-	-	-	-	9.7222	0.6843	-	-	-	-	18.0556	0.8885	-	-
-	-	-	-	-	-	-	-	9.7222	0.6843	-	-	-	-	18.0556	0.8885	-	-
-	-	-	-	-	-	-	-	0.8230	0.1136	0.4115	0.0805	-	-	4.1152	0.2498	-	-
-	-	-	-	-	-	-	-	5.1546	0.4400	-	-	-	-	6.1856	0.4794	-	-
-	-	-	-	-	-	-	-	6.6667	0.5154	-	-	-	-	2.2222	0.3045	-	-
0.5464	0.1068	-	-	-	-	-	-	3.8251	0.2779	-	-	-	-	3.2787	0.2580	-	-
-	-	-	-	1.6260	0.1580	-	-	0.8130	0.1122	-	-	-	-	10.5691	0.3842	0.4065	0.0795
-	-	-	-	-	-	-	-	3.5928	0.2823	-	-	6.5868	0.3762	-	-	-	-
1.9737	0.1564	-	-	1.6447	0.1430	-	-	5.2632	0.2510	0.9868	0.1111	-	-	9.5395	0.3302	-	-
-	-	-	-	-	-	-	-	11.1940	0.5338	2.9851	0.2881	-	-	2.9851	0.2881	-	-
-	-	-	-	-	-	-	-	-	-	0.5814	0.1136	-	-	11.0465	0.4685	-	-
-	-	-	-	-	-	-	-	2.8269	0.1931	-	-	-	-	9.8940	0.3479	-	-
0.8097	0.1118	-	-	2.0243	0.1756	-	-	6.0729	0.2979	0.8097	0.1118	-	-	12.5506	0.4132	-	-

Appendix 10d: Constrained cluster analysis for SP Core 3



Appendix 11a: Protozoan raw data for Chem-Core

Depth (cm)	*C. aculeata*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*	*P. compressa*	*C. tricuspis*	*D. protaeiformis*
0	-	2	9	1	1	0	103	21
7	32	3	39	-	19	1	1058	66
13	13	4	74	-	3	4	103	27
20	36	-	77	4	11	6	94	27
25	20	5	11	-	-	2	51	22

D. bidens	*D. corona*	*D. fragosa*	*D. urceolata*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total Protozoans	Protozoans/ml	SDI
-	-	-	-	3	2	-	211	353	141.2	0.8
2	1	1	5	44	2	-	53	1326	530.4	0.8
-	1	-	1	13	7	17	106	373	149.2	1.7
-	1	1	-	32	14	18	53	374	149.6	2.0
-	-	-	-	11	8	9	28	167	66.8	1.9

Appendix 11b: Total protozoans per ml for Chem-Core

Depth (cm)	*C. aculeata*	*nstricta aeropn*	*stricta constr*	*C. constricta spinosa*	*L. spiralis*	*P. compressa*
0	-	1	4	-	-	-
7	13	1	16	-	8	-
13	5	2	30	-	1	2
20	14	-	31	2	4	2
25	8	2	4	-	-	1

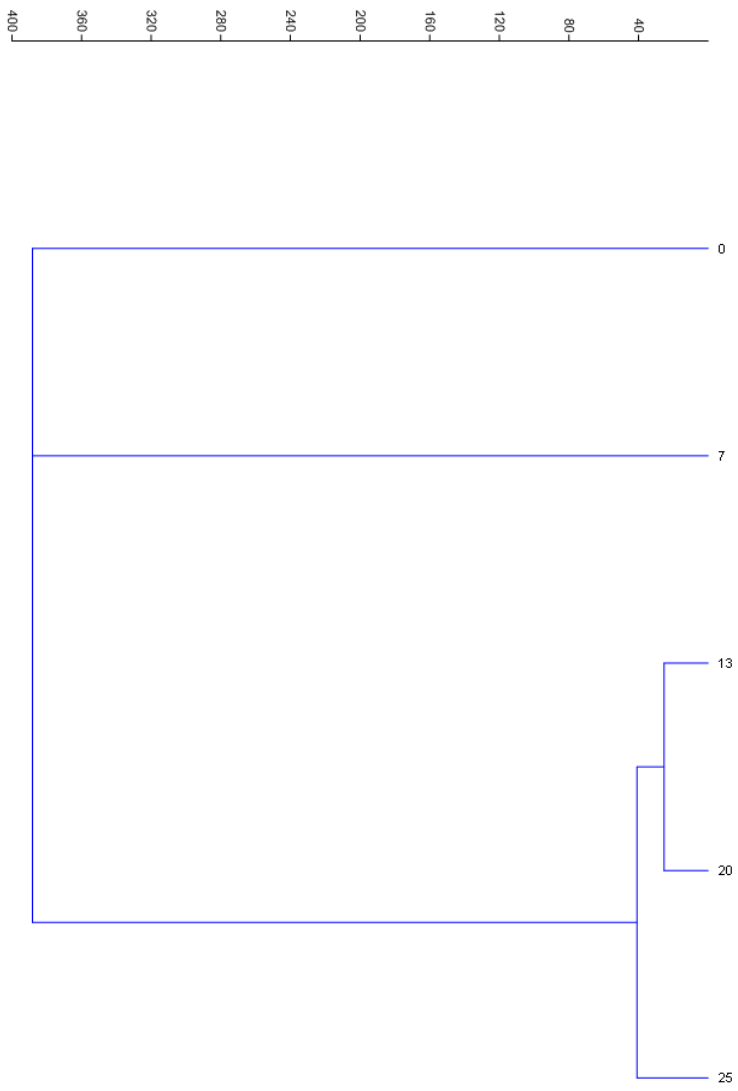
C. tricuspis	*D. protaeiformis*	*D. bidens*	*D. corona*	*D. fragosa*	*D. urceolata*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total
41	8	-	-	-	-	1	1	-	84	70
423	26	1	-	-	2	18	1	-	21	565
41	11	-	-	-	-	5	3	7	42	154
38	11	-	-	-	-	13	6	7	21	186
20	9	-	-	-	-	4	3	4	11	79

Appendix 11c: Protozoan percent abundance and percent error in Chem-Core

	C. aculeata		*C. constricta aerophila*		*C. constricta constricta*		*C. constricta spinosa*		*L. spiralis*		*P. compressa*	
Depth (cm)	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0	-	-	1.4085	0.1938	6.3380	0.4007	0.7042	0.1412	0.7042	0.1412	-	-
7	2.5137	0.0860	0.2357	0.0266	3.0636	0.0947	-	-	1.4925	0.0666	0.0786	0.0154
13	4.8689	0.2582	1.4981	0.1457	27.7154	0.5369	-	-	1.1236	0.1264	1.4981	0.1457
20	11.2150	0.3452	-	-	23.9875	0.4671	1.2461	0.1214	3.4268	0.1990	1.8692	0.1482
25	14.3885	0.5835	3.5971	0.3096	7.9137	0.4488	-	-	-	-	1.4388	0.1980

C. tricuspis		*D. protaeiformis*		*D. bidens*		*D. corona*		*D. fragosa*		*D. urceolata*		*D. oblonga*		*D. bacillifera*		*D. amphora*	
%A	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
72.5352	0.7342	14.7887	0.5839	-	-	-	-	-	-	-	-	2.1127	0.2365	1.4085	0.1938	-	-
83.1108	0.2058	5.1846	0.1218	0.1571	0.0218	0.0786	0.0154	0.0786	0.0154	0.3928	0.0344	3.4564	0.1003	0.1571	0.0218	-	-
38.5768	0.5839	0.1011	0.3616	-	-	0.3745	0.0733	-	-	0.3745	0.0733	4.8689	0.2582	2.6217	0.1917	6.3670	0.2971
29.2835	0.4978	8.4112	0.3036	-	-	0.3115	0.0610	0.3115	0.0610	-	-	9.9688	0.3277	4.3614	0.2234	5.6075	0.2517
36.6906	0.8012	15.8273	0.6068	-	-	-	-	-	-	-	-	7.9137	0.4484	5.7554	0.3872	0.0647	0.4092

Appendix 11d: Constrained cluster analysis for Chem-Core



Appendix 12a: Protozoan raw data for selected surface sediments

Sample Name	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta 'aerophila'*	*C. constricta 'constricta'*	*C. constricta 'spinosa'*	*L. spiralis*	*H. sphagni*	*P. compressa*	*C. tricuspis*	*D. protaeiformis*
SP Core 2	2	21	1	13	51	-	3	-	40	48	18
SP Core 3	-	4	-	5	19	-	1	-	18	12	3
Chem-core	-	-	-	2	9	1	1	-	-	103	21
SG 1	3	25	3	5	30	-	3	-	29	57	4
SG 2	4	5	2	-	4	-	-	-	-	70	17
SG 3	1	3	-	4	1	-	3	-	1	7	8

D. bidens	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total Protozoa	Protozoa/ml	Total theca	SDI
3	7	-	10	-	158	-	-	7	382	152.8	375	1.9
-	4	1	8	-	242	-	-	6	323	129.2	317	1
-	-	-	-	-	3	2	-	211	353	141.2	142	0.9
2	5	-	-	5	208	-	-	19	398	159.2	379	1.6
-	1	-	-	1	10	-	-	116	230	92	114	1.3
-	5	-	-	2	133	-	-	1	169	67.6	168	0.9

Appendix 12b: Total protozoans per ml for selected surface sediments

Sample Name	*A. vulgaris*	*C. aculeata*	*C. discoides*	*C. constricta aerophila*	*C. constricta constricta*	*C. constricta spinosa*	*L. spiralis*
SP Core 2	1	6	-	4	15	-	1
SP Core 3	-	2	-	2	8	-	-
Chem-core	-	-	-	1	4	-	-
SG 1	1	10	1	2	12	-	1
SG 2	2	2	1	-	2	-	-
SG 3	-	1	-	2	-	-	1

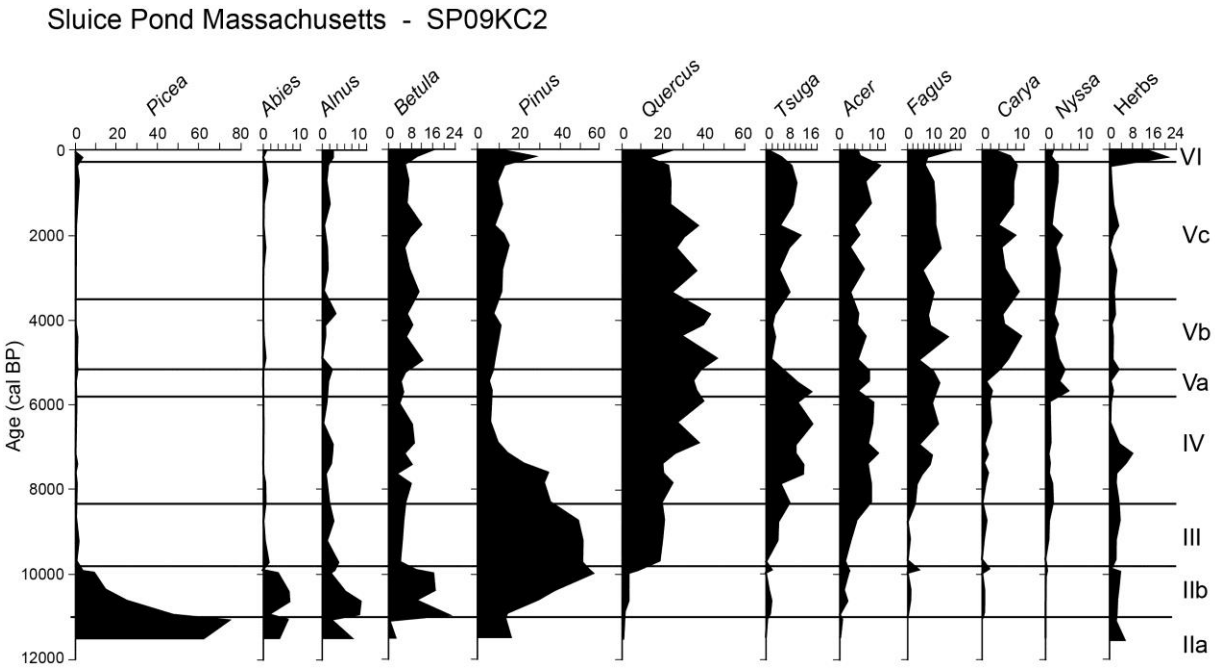
P. compressa	*C. tricuspis*	*D. protaeiformis*	*D. bidens*	*D. corona*	*D. urens*	*D. urceolata*	*D. globula*	*D. oblonga*	*D. bacillifera*	*D. amphora*	*C. cratera*	Total theca/ml
11	14	5	1	2	-	3	-	45	-	-	2	107
7	5	1	-	2	-	3	-	97	3	-	2	129
-	41	8	-	-	-	-	-	1	-	-	84	55
12	23	2	1	2	-	-	2	83	-	-	8	152
-	28	7	-	-	-	-	-	4	-	-	46	45
-	3	3	-	2	-	-	1	53	-	-	**0**	**66**

Appendix 12c: Protozoan percent abundance and percent error in selected surface sediments

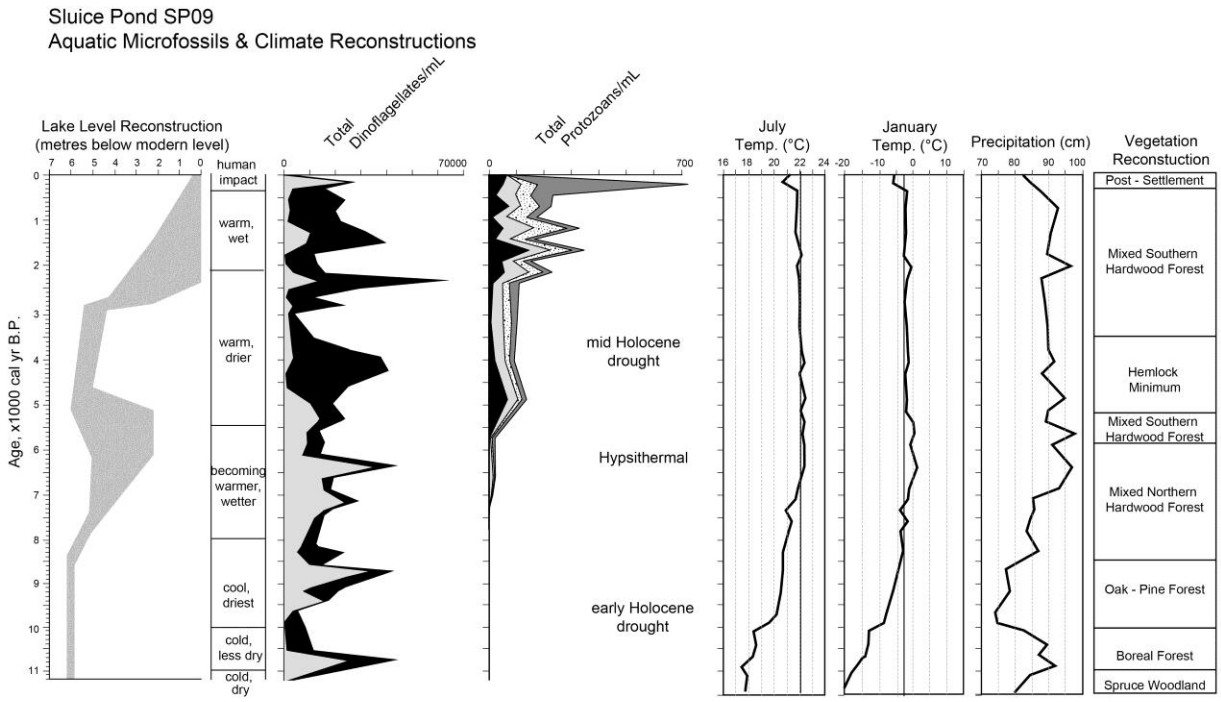
	A. vulgaris		*C. aculeata*		*C. discoides*		*C. constricta 'aerophila'*		*C. constricta 'constri*		*C. constricta 'spinosa*		*L. spiralis*		*P. compressa*		*C. tricuspis*	
Sample Name	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
SP Core 2	0.0052	0.0724	5.4974	0.2286	0.2618	0.0512	3.4031	0.1818	13.3508	0.3411	-	-	0.7853	0.0885	10.4712	0.3070	1.5079	0.3323
SP Core 3	-	-	1.2384	0.1206	-	-	1.5480	0.1346	5.8824	0.2566	-	-	0.3096	0.0606	5.5728	0.2502	3.7152	0.2063
Chem-core	-	-	-	-	-	-	0.5666	0.0783	2.5496	0.1644	0.2833	0.0554	0.2833	0.0554	-	-	29.1785	0.4742
SG 1	0.7538	0.0850	6.2814	0.2384	0.7538	0.0850	1.2563	0.1094	7.5377	0.2594	-	-	0.7538	0.0850	7.2864	0.2554	14.3216	0.3441
SG 2	1.7391	0.1689	2.1739	0.1885	0.8696	0.1200	-	-	1.7391	0.1689	-	-	-	-	-	-	30.4348	0.5947
SG 3	0.5917	0.1156	1.7751	0.1991	-	-	2.3669	0.2292	0.5917	0.1156	-	-	1.7751	0.1991	0.5917	0.1156	3.0435	0.2590

D. protaeiformis		*D. bidens*		*D. corona*		*D. urens*		*D. urceolata*		*D. globula*		*D. oblonga*		*D. bacillifera*		*D. amphora*	
%A	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**	**%A**	**E**
0.0471	0.2125	0.7853	0.0885	1.8325	0.1345	-	-	2.6178	0.1601	-	-	41.3613	0.4939	-	-	-	-
0.9288	0.1001	-	-	0.0124	0.1206	0.3096	0.0606	2.4768	0.1695	-	-	74.9226	0.4727	-	-	-	-
5.9490	0.2468	-	-	-	-	-	-	-	-	-	-	0.8499	0.0958	0.5666	0.0783	-	-
1.0050	0.0980	0.5025	0.0695	1.2563	0.1094	-	-	-	-	1.2563	0.1094	52.2613	0.4907	-	-	-	-
7.3913	0.3381	-	-	0.4348	0.0850	-	-	-	-	0.4348	0.0850	43.4783	0.6407	-	-	-	-
4.7337	0.3203	-	-	2.1739	0.2199	-	-	-	-	1.1834	0.1630	78.6982	0.6173	-	-	-	-

Appendix 13: Pollen analysis for SP09 (Analyst: Dr. F McCarthy)



Relative abundance of major pollen taxa in the basin core (SP09 KC2) from Sluice Pond plotted against age using the age model of Hubeny et al., in press for basin core (raw pollen data below), showing the typical zonation for southern New England modified from Foster and Zebryk (1993). The vegetation succession from spruce woodland (zone IIa) through boreal forest (zone IIb), oak-pine forest (zone III), a rise in hemlock (*Tsuga*) producing a northern hardwood (e.g., maple/ *Acer*, beech/ *Fagus*)-dominated mixed forest (zone IV) through southern hardwood (e.g., hickory/ *Carya*, tupelo- *Nyssa*)-dominated mixed forest (zone V) records Holocene climate change. Drought-tolerant taxa, such as pine (*Pinus*), oak (*Quercus*), and herb pollen identify arid intervals during the early Holocene and contributing to the hemlock (*Tsuga*) minimum (~ 5.4 – 3.5 ka using the age model, compares well with estimates of ~5.3 – 3.2 ka for New England (Newby et al. 2000; Shuman et al. 2001; Webb et al. 2004).



Reconstructions of paleoclimate using pollen-derived transfer functions of Bartlein and Whitlock (1993) for the closest calibration region (45 – 55^{o}N, 75 – 87^{o}W) illustrate gradual warming during the dry early Holocene with a sharp increase ca. 10ka, peak warmth and moisture during the mid-Holocene hypsithermal (ca. 7.5 – 5.5 ka) allowing the eventual establishment of the southern hardwood-dominated mixed forest that persisted through the hemlock minimum. Modern values of mean July and January temperatures in Salem, MA are shown using vertical grey lines, but the transfer function reconstructed much lower precipitation in modern sediments, so the modern value is not shown. Aquatic microfossils record limnological changes, with high algal productivity (dinoflagellate cysts- grey =*Peridinium wisconsinense*, black = eutrophic dinoflagellate species) corresponding to warmer and drier conditions, and protozoan assemblages recording changes in dissolved oxygen, with abundant *Cucurbitella tricuspis* (dark grey) and *Codonella cratera* (black) recording highly eutrophic conditions leading to bottom water anoxia ca. 5.1 ka and in the post-settlement zone. Arid intervals correlate with low lake levels throughout southern New England (reproduced from Webb et al. 2004).

Age (cal yBP)	**1**	**160**	**365**	**733**	**1277**	**1772**	**2284**	**2807**	**3336**	**3865**	
Depth (cm)		**1**	**10**	**20**	**40**	**60**	**80**	**100**	**120**	**140**	**160**
1*Picea*		1	6	1	5	2.5	1		2		
2*Abies*		1		1	2			1			
4Cupressaceae	3										
5*Pinus*		21	49	25	25	35	17	37	43	22.5	25
6*Betula*		30	18	11	18	19	22	14	26	20	21
8*Fraxinus*		8	18	20	18	23	20	30	36	15	18
10*Quercus*		46	23	43	60	70	69	52	128	45	135
11*Ulmus*		13	3	6	7	4	6	4	8	9	5
12*Ostrya*		1	2	5	4	6	1	6	6	3	9
13*Salix*		5	1		1			1	1		
14*Acer*		9	9	20	17	20	7	6	22	5	15
17*Tilia*		1	1			1			1		2
18*Juglans*					1		1			2	1
20*Carya*		5	12	16	19	21	7	11	19	16	5
21*Tsuga*		2	9	17	27	26	9	19	16	15	14
24*Fagus*		31	13	13	25	30	20	31	20	18	35
23*Nyssa*		4	3	6	8	6	3	6	13	6	7
25*Liquidambar*											
26*Castanea*		1				1			1		1
30*Alnus*		6	5	3	3	6	1	3	5	1	5
31*Corylus*		1	1				3	2		3	1
35*Vitis*									1		
36Ericaceae					1	1	1				
42Gramineae		8	16	1		1	2		5		1
44*Artemisia*		2	2						2		
45*Ambrosia*		6	8	1			1		2		2
49Compositae		1		1	1						
51Chenopodiaceae	1	1			1	1			1	1	
57*Plantago*		2	1								
56*Rumex*		4	3								
80Cyperaceae	2	5		1	2	3			2	2	
84other aquatics	3	1	1	1						1	
70trilete spore	2	1	2	2	1	3		2	4	2	
73*Dryopteris*	2							1		1	
Other			1	3	5	7	11	4	13	4	6

Age	4129	4391	4937	5194	5449	5701	5950	6440	6932	7156	7390	7622
Depth	170	180	200	210	220	230	240	260	280	290	300	310
1		2	1	1			1				2	
2			1							0.5		
4			1							1		
5	26	23.5	18.5	11	10.5	16.5	11.5	15	23	32	54	78
6	19	14	26	9	9	11	7	20	20	13	20	6
8	28	17	15	11	18	10	21	21	23	24	17	18
10	91	66	100	57	68	77	69	61	82	57	47	46
11	1	3	5	3	6	4	3	3	3	7	8	3
12	4	4	2	2	1	1	2	1	2	4		5
13			1	1		3			1	1		1
14	10	15	10	11	15	10	15	20	17	22	17	17
17	1	1					1	1		2		1
18		1	1		1	1						
20	12	21	13	6	2	5	3	5	1	3	1	3
21	5	7	4	9	22	34	19	38	23	23	31	29
24	19	34	10	14	24	18	16	24	13	20	20	12
23	7	5	7	7	7	12	2	3	3	2	3	2
25									1			
26	1	1										
30	2	2		4	3	2	2		6	6	6	2
31										1	1	
35					1							
36												
42		2	2	2		2	1	3		7	4	2
44			1	1	1					1	1	
45				1		1		1	4	3	6	4
49				1					2			
51								2		1		
36												
57												
56												
80	2	1						1	1	6	3	
84			1	2				1	1			
70	1	1		1	3	3	3	4	2	5	2	2
73								1		1	2	1
Other	3	9	2	1	13	12	1	12	1	5	11	2

Age	7860	8307	8715	9202	9695	9895	10060	10373	10619	10850	11052	11400
Depth	320	340	360	380	400	410	420	430	440	448	458	471
1	1			3	1	8	19	25.5	51	93	152	69
2	1	1		1	3		9	12.5	15	3	13.5	5
4		3										
5	78	74	112	128	111	133	130	70	63	30	30	19
6	19	13	12	12	9	24	37	31	21	43	1	3
8	22	23	11	14	18	10	2	6	5			2
10	60	41	46	49	40	19	8	6	7	2	2	1
11	3		4	3	6	4	3	3	4		1	2
12	3	5	3	7	10	5	2	4	4	1	1	1
13	1	1		1					1	1		1
14	19	17	10	7	3	6	5	2	4		1	
17								1				
18		1	1									
20	2		1	1		4		1	1	1		
21	12	17	10	10	3	5		2	4	3	1	
24	8	7	6	2	1	10	1	3	2			
23	4	4	2	2		1						
25												
26												
30	3	4	5	3	9	8	5	12	22	20	5	9
31	1		1	2		1	6	1		1		2
35												
36						1	2	3		1		
42	1	1	3	2	2	1	3	3	3	2	3	1
44					1		1				1	
45	3	4	4			1	1	1				
49	1	2	1				1	1			2	3
51				1			1					
36												
57												
56												
80	1	3		3	2		2	2	3	3		3
84								1				
70	5	2	2	2	4	2	5	6	17	1	4	1
73			1		2			1	2			
Other	2	1	1	4	1	4	2	6	6	3	1	1

Appendix 14: Elemental analysis

E3 Laboratories Inc.
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CERTIFICATE OF ANALYSIS

Brock University-Earth Sciences Dept.
Matea Drljepan
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Tel: 905-688-5550 Fax:
Email:

Work Order No.:2502491
Received : 2013-02-01
PO Number:
Reported: 2013-02-08
Project Name:
Chain of Custody No.: 24691

Client Sample ID	Sample Date	Lab ID	Parameter	Result	Unit	RDL	Date Analyzed	Method
Sluice Pond 0cm	2013-01-30	**318411**	Arsenic	43.8	mg/Kg	2.13	2013-02-06	Hydr/Vap Gen EPA3005
			Cadmium	8.0	mg/Kg	1.1	2013-02-06	EPA 3050B
			Chromium	89.4	mg/Kg	0.64	2013-02-06	EPA 3050B
			Cobalt	34.1	mg/Kg	0.64	2013-02-06	EPA 3050B
			Copper	169	mg/Kg	0.64	2013-02-06	EPA 3050B
			Lead	938	mg/Kg	0.64	2013-02-06	EPA 3050B
			Mercury	1.42	mg/Kg	0.32	2013-02-06	Hydr/Vap Gen EPA3005
			Molybdenum	4.37	mg/Kg	0.64	2013-02-06	EPA 3050B
			Nickel	180	mg/Kg	0.64	2013-02-06	EPA 3050B
			NO2 (Nitrite)	0.704	mg/L	0.004	2013-02-05	APHA 4500/HACH 8503m
			NO3 (Nitrate)	<0.04	mg/L	0.04	2013-02-05	APHA 4500/HACH 8171m
			Selenium	3.52	mg/Kg	2.13	2013-02-06	Hydr/Vap Gen EPA3005
			TP	2090	mg/Kg	0	2013-02-06	EPA 3050B
			Zinc	964	mg/Kg	4.3	2013-02-06	EPA 3050B
Sluice Pond 7cm	2013-01-30	**318412**	Arsenic	2.12	mg/Kg	1.56	2013-02-06	Hydr/Vap Gen EPA3005
			Cadmium	1.98	mg/Kg	0.78	2013-02-06	EPA 3050B
			Chromium	64.4	mg/Kg	0.47	2013-02-06	EPA 3050B
			Cobalt	5.70	mg/Kg	0.47	2013-02-06	EPA 3050B
			Copper	5.54	mg/Kg	0.47	2013-02-06	EPA 3050B
			Lead	12.5	mg/Kg	0.47	2013-02-06	EPA 3050B
			Mercury	<0.23	mg/Kg	0.23	2013-02-06	Hydr/Vap Gen EPA3005
			Molybdenum	1.58	mg/Kg	0.47	2013-02-06	EPA 3050B
			Nickel	71.9	mg/Kg	0.47	2013-02-06	EPA 3050B
			NO2 (Nitrite)	0.304	mg/L	0.004	2013-02-05	APHA 4500/HACH 8503m
			NO3 (Nitrate)	<0.04	mg/L	0.04	2013-02-05	APHA 4500/HACH 8171m
			Selenium	2.06	mg/Kg	1.56	2013-02-06	Hydr/Vap Gen EPA3005
			TP	582	mg/Kg	0	2013-02-06	EPA 3050B
			Zinc	72.3	mg/Kg	3.1	2013-02-06	EPA 3050B
Sluice Pond 13cm	2013-01-30	**318413**	Arsenic	<2.33	mg/Kg	2.33	2013-02-06	Hydr/Vap Gen EPA3005
			Cadmium	2.5	mg/Kg	1.2	2013-02-06	EPA 3050B
			Chromium	54.0	mg/Kg	0.70	2013-02-06	EPA 3050B
			Cobalt	3.63	mg/Kg	0.70	2013-02-06	EPA 3050B
			Copper	7.03	mg/Kg	0.70	2013-02-06	EPA 3050B

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CERTIFICATE OF ANALYSIS

Brock University-Earth Sciences Dept.
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Work Order No.:2502491
Received : 2013-02-01
PO Number:
Reported: 2013-02-08
Project Name:
Chain of Custody No.: 24691

Client Sample ID	Sample Date	Lab ID	Parameter	Result	Unit	RDL	Date Analyzed	Method
Sluice Pond 13cm	2013-01-30	**318413**	Lead	3.15	mg/Kg	0.70	2013-02-06	EPA 3050B
			Mercury	<0.35	mg/Kg	0.35	2013-02-06	Hydr/Vap Gen EPA3005
			Molybdenum	1.49	mg/Kg	0.70	2013-02-06	EPA 3050B
			Nickel	69.5	mg/Kg	0.70	2013-02-06	EPA 3050B
			NO2 (Nitrite)	0.275	mg/L	0.004	2013-02-05	APHA 4500/HACH 8503m
			NO3 (Nitrate)	<0.04	mg/L	0.04	2013-02-05	APHA 4500/HACH 8171m
			Selenium	2.68	mg/Kg	2.33	2013-02-06	Hydr/Vap Gen EPA3005
			TP	464	mg/Kg	0	2013-02-06	EPA 3050B
			Zinc	76.3	mg/Kg	4.7	2013-02-06	EPA 3050B
Sluice Pond 20cm	2013-01-30	**318414**	Arsenic	<2.86	mg/Kg	2.86	2013-02-06	Hydr/Vap Gen EPA3005
			Cadmium	2.4	mg/Kg	1.4	2013-02-06	EPA 3050B
			Chromium	52.6	mg/Kg	0.86	2013-02-06	EPA 3050B
			Cobalt	3.37	mg/Kg	0.86	2013-02-06	EPA 3050B
			Copper	7.40	mg/Kg	0.86	2013-02-06	EPA 3050B
			Lead	2.82	mg/Kg	0.86	2013-02-06	EPA 3050B
			Mercury	<0.43	mg/Kg	0.43	2013-02-06	Hydr/Vap Gen EPA3005
			Molybdenum	1.57	mg/Kg	0.86	2013-02-06	EPA 3050B
			Nickel	69.5	mg/Kg	0.86	2013-02-06	EPA 3050B
			NO2 (Nitrite)	0.324	mg/L	0.004	2013-02-05	APHA 4500/HACH 8503m
			NO3 (Nitrate)	<0.04	mg/L	0.04	2013-02-05	APHA 4500/HACH 8171m
			Selenium	2.92	mg/Kg	2.86	2013-02-06	Hydr/Vap Gen EPA3005
			TP	429	mg/Kg	0	2013-02-06	EPA 3050B
			Zinc	77.0	mg/Kg	5.7	2013-02-06	EPA 3050B
Sluice Pond 25cm	2013-01-30	**318415**	Arsenic	<2.00	mg/Kg	2.00	2013-02-06	Hydr/Vap Gen EPA3005
			Cadmium	2.4	mg/Kg	1.0	2013-02-06	EPA 3050B
			Chromium	58.5	mg/Kg	0.60	2013-02-06	EPA 3050B
			Cobalt	3.87	mg/Kg	0.60	2013-02-06	EPA 3050B
			Copper	7.64	mg/Kg	0.60	2013-02-06	EPA 3050B
			Lead	2.70	mg/Kg	0.60	2013-02-06	EPA 3050B
			Mercury	<0.30	mg/Kg	0.30	2013-02-06	Hydr/Vap Gen EPA3005
			Molybdenum	1.76	mg/Kg	0.60	2013-02-06	EPA 3050B
			Nickel	72.3	mg/Kg	0.60	2013-02-06	EPA 3050B
			NO2 (Nitrite)	0.435	mg/L	0.004	2013-02-05	APHA 4500/HACH 8503m

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SS#4, 360 York Rd., Unit 10, Niagara-on-the-Lake, Ontario L0S 1J0
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CERTIFICATE OF ANALYSIS

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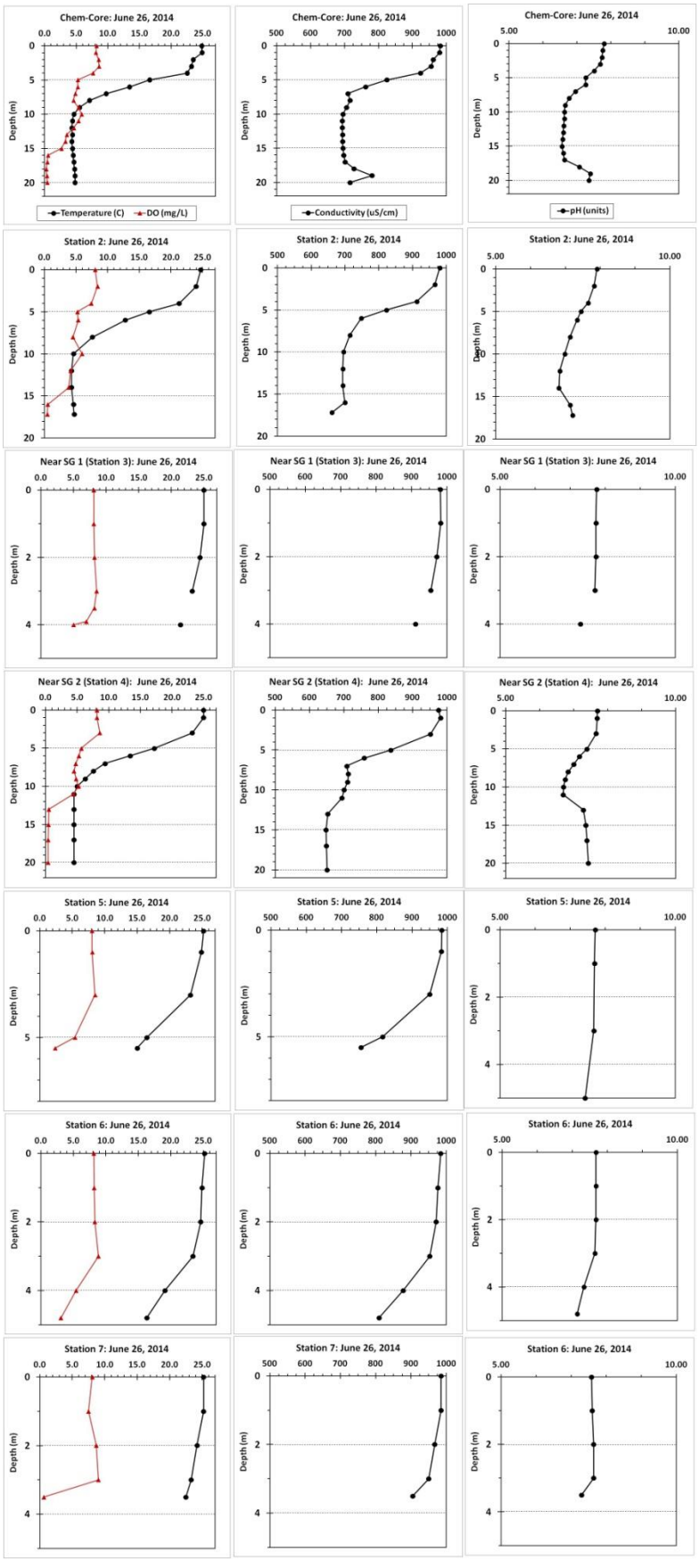
Client Sample ID	Sample Date	Lab ID	Parameter	Result	Unit	RDL	Date Analyzed	Method
Sluice Pond 25cm	2013-01-30	**318415**	NO3 (Nitrate)	<0.04	mg/L	0.04	2013-02-05	APHA 4500/HACH 8171m
			Selenium	2.47	mg/Kg	2.00	2013-02-06	Hydr/Vap Gen EPA3005
			TP	386	mg/Kg	0	2013-02-06	EPA 3050B
			Zinc	76.9	mg/Kg	4.0	2013-02-06	EPA 3050B

Appendix 15: DO, conductivity, temperature, pH parameters

Station	Date	Northing	Westing	Depth (m)	Temp (°C)	Conductivity (uScm	pH (units)	DO (mg/L)
1	26-Jun-14	42°29.307'	70°57.834'	0	24.8	982	7.80	8.20
1	26-Jun-14	42°29.307'	70°57.834'	1	24.9	980	7.76	8.10
1	26-Jun-14	42°29.307'	70°57.834'	2	23.5	962	7.73	8.52
1	26-Jun-14	42°29.307'	70°57.834'	3	23.2	955	7.68	8.61
1	26-Jun-14	42°29.307'	70°57.834'	4	22.5	924	7.50	7.65
1	26-Jun-14	42°29.307'	70°57.834'	5	16.6	825	7.25	5.20
1	26-Jun-14	42°29.307'	70°57.834'	6	13.4	762	7.25	5.25
1	26-Jun-14	42°29.307'	70°57.834'	7	9.7	710	6.95	4.82
1	26-Jun-14	42°29.307'	70°57.834'	8	7.1	716	6.77	4.56
1	26-Jun-14	42°29.307'	70°57.834'	9	5.5	706	6.65	5.35
1	26-Jun-14	42°29.307'	70°57.834'	10	4.6	696	6.63	5.86
1	26-Jun-14	42°29.307'	70°57.834'	11	4.4	693	6.63	5.32
1	26-Jun-14	42°29.307'	70°57.834'	12	4.3	693	6.61	4.62
1	26-Jun-14	42°29.307'	70°57.834'	13	4.4	694	6.60	3.48
1	26-Jun-14	42°29.307'	70°57.834'	14	4.3	694	6.58	3.25
1	26-Jun-14	42°29.307'	70°57.834'	15	4.4	696	6.56	2.64
1	26-Jun-14	42°29.307'	70°57.834'	16	4.5	698	6.60	0.52
1	26-Jun-14	42°29.307'	70°57.834'	17	4.6	702	6.63	0.42
1	26-Jun-14	42°29.307'	70°57.834'	18	4.7	728	7.07	0.23
1	26-Jun-14	42°29.307'	70°57.834'	19	4.8	781	7.39	0.37
1	26-Jun-14	42°29.307'	70°57.834'	20	4.8	716	7.35	0.40
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
2	26-Jun-14	42°29.356'	70°57.824'	0	24.7	980	7.90	8.02
2	26-Jun-14	42°29.356'	70°57.824'	2	24.0	966	7.82	8.40
2	26-Jun-14	42°29.356'	70°57.824'	4	21.3	912	7.65	7.42
2	26-Jun-14	42°29.356'	70°57.824'	5	16.6	822	7.45	5.20
2	26-Jun-14	42°29.356'	70°57.824'	6	12.8	748	7.33	5.35
2	26-Jun-14	42°29.356'	70°57.824'	8	7.6	714	7.13	4.50
2	26-Jun-14	42°29.356'	70°57.824'	10	4.6	695	6.98	5.95
2	26-Jun-14	42°29.356'	70°57.824'	12	4.3	693	6.84	4.15
2	26-Jun-14	42°29.356'	70°57.824'	14	4.3	693	6.81	3.84
2	26-Jun-14	42°29.356'	70°57.824'	16	4.6	700	7.13	0.53
2	26-Jun-14	42°29.356'	70°57.824'	17.2	4.7	661	7.20	0.45
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
3	26-Jun-14	42°29.272'	70°57.714'	0	25.0	981	7.76	8.10
3	26-Jun-14	42°29.272'	70°57.714'	1	25.0	982	7.74	8.12
3	26-Jun-14	42°29.272'	70°57.714'	2	24.4	971	7.74	8.23
3	26-Jun-14	42°29.272'	70°57.714'	3	23.2	954	7.71	8.57
3	26-Jun-14	42°29.272'	70°57.714'	3.5				8.20
3	26-Jun-14	42°29.272'	70°57.714'	3.9				7.00
3	26-Jun-14	42°29.272'	70°57.714'	4	21.4	911	7.30	5.00
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
4	26-Jun-14	42°29.286'	70°57.761'	0	25.0	975	7.70	8.14
4	26-Jun-14	42°29.286'	70°57.761'	1	25.0	981	7.69	8.15
4	26-Jun-14	42°29.286'	70°57.761'	3	23.2	952	7.66	8.57
4	26-Jun-14	42°29.286'	70°57.761'	5	17.2	836	7.39	5.65
4	26-Jun-14	42°29.286'	70°57.761'	6	13.4	759	7.17	5.27
4	26-Jun-14	42°29.286'	70°57.761'	7	9.4	708	7.01	4.79
4	26-Jun-14	42°29.286'	70°57.761'	8	7.6	713	6.84	4.50
4	26-Jun-14	42°29.286'	70°57.761'	9	6.3	711	6.75	4.85
4	26-Jun-14	42°29.286'	70°57.761'	10	5.0	700	6.70	5.20
4	26-Jun-14	42°29.286'	70°57.761'	11	4.5	694	6.69	4.45
4	26-Jun-14	42°29.286'	70°57.761'	13	4.5	653	7.29	0.55
4	26-Jun-14	42°29.286'	70°57.761'	15	4.5	648	7.36	0.46
4	26-Jun-14	42°29.286'	70°57.761'	17	4.5	649	7.39	0.44
4	26-Jun-14	42°29.286'	70°57.761'	20	4.5	651	7.43	0.44
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
5	26-Jun-14	42°29.264'	70°57.745'	0	25.2	984	7.72	8.05
5	26-Jun-14	42°29.264'	70°57.745'	1	24.9	983	7.70	8.07
5	26-Jun-14	42°29.264'	70°57.745'	3	23.2	950	7.68	8.51
5	26-Jun-14	42°29.264'	70°57.745'	5	16.5	817	7.43	5.40
5	26-Jun-14	42°29.264'	70°57.745'	5.5	15.0	755	7.24	2.40
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
6	26-Jun-14	42°29.369'	70°57.968'	0	25.2	984	7.67	8.14
6	26-Jun-14	42°29.369'	70°57.968'	1	24.8	976	7.67	8.23
6	26-Jun-14	42°29.369'	70°57.968'	2	24.6	971	7.67	8.34
6	26-Jun-14	42°29.369'	70°57.968'	3	23.4	953	7.64	8.87
6	26-Jun-14	42°29.369'	70°57.968'	4	19.1	878	7.33	5.45
6	26-Jun-14	42°29.369'	70°57.968'	4.8	16.3	809	7.14	3.10
Station	**Date**	**Northing**	**Westing**	**Depth (m)**	**Temp (°C)**	**Conductivity (uScm**	**pH (units)**	**DO (mg/L)**
7	26-Jun-14	42°29.412'	70°58.035'	0	25.2	985	7.58	8.07
7	26-Jun-14	42°29.412'	70°58.035'	1	25.2	985	7.60	7.50
7	26-Jun-14	42°29.412'	70°58.035'	2	24.2	967	7.64	8.72
7	26-Jun-14	42°29.412'	70°58.035'	3	23.3	950	7.64	9.01
7	26-Jun-14	42°29.412'	70°58.035'	3.5	22.5	905	7.30	0.65


Chem-Core: June 26, 2014
Depth (m)
Temperature (C)
DO (mg/L)
Chem-Core: June 26, 2014
Depth (m)
Conductivity (uS/cm)
Chem-Core: June 26, 2014
Depth (m)
pH (units)
Station 2: June 26, 2014
Depth (m)
Station 2: June 26, 2014
Depth (m)
Station 2: June 26, 2014
Depth (m)
Near SG 1 (Station 3): June 26, 2014
Depth (m)
Near SG 1 (Station 3): June 26, 2014
Depth (m)
Near SG 1 (Station 3): June 26, 2014
Depth (m)
Near SG 2 (Station 4): June 26, 2014
Depth (m)
Near SG 2 (Station 4): June 26, 2014
Depth (m)
Near SG 2 (Station 4): June 26, 2014
Depth (m)
Station 5: June 26, 2014
Depth (m)
Station 5: June 26, 2014
Depth (m)
Station 5: June 26, 2014
Depth (m)
Station 6: June 26, 2014
Depth (m)
Station 6: June 26, 2014
Depth (m)
Station 6: June 26, 2014
Depth (m)
Station 7: June 26, 2014
Depth (m)
Station 7: June 26, 2014
Depth (m)
Station 6: June 26, 2014
Depth (m)

Appendix 16: Additional chronological data

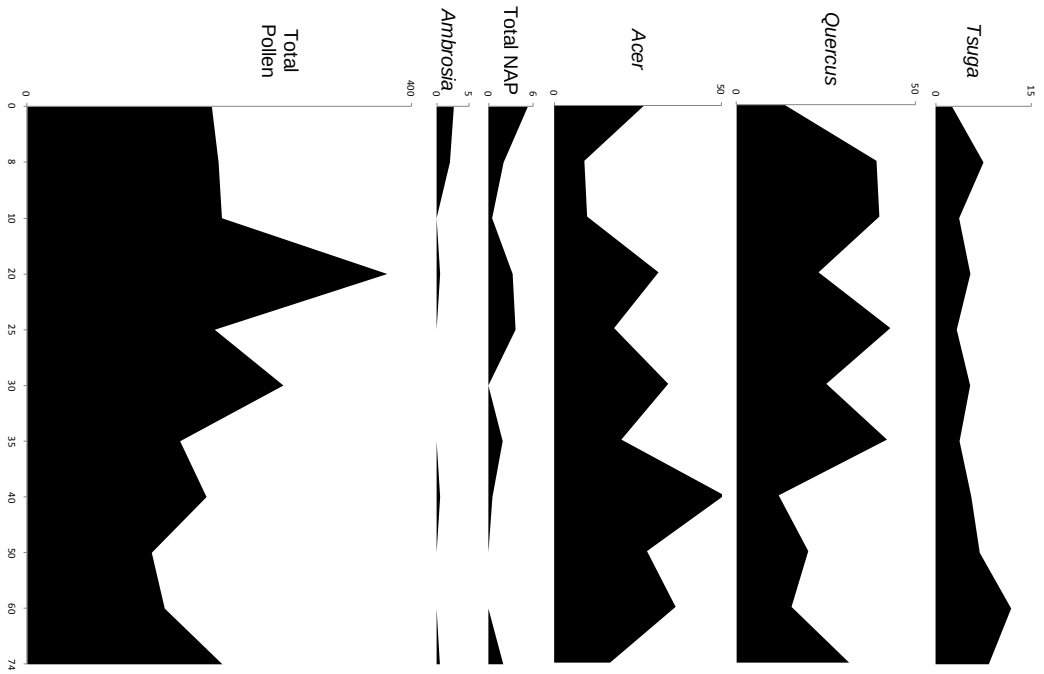


Figure 5.1: Pollen data for SP Core 2.

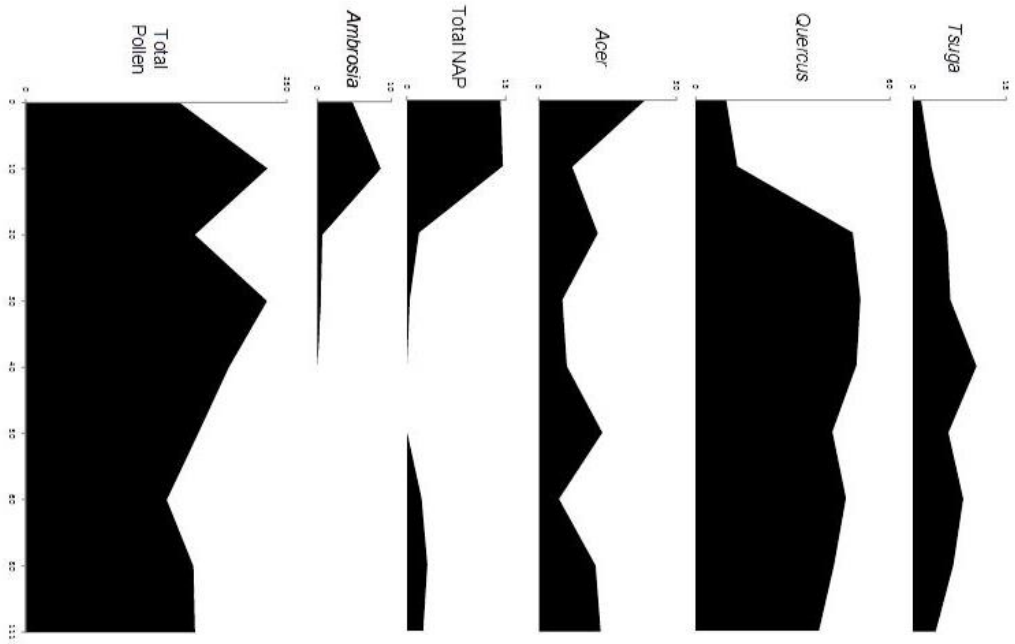


Figure 5.2: Pollen data for SP Core 3.

Figure 6.1: Common forest types in the United States. A domination of oak-hickory is present in Massachusetts forests (https://store.usgs.gov/yimages/PDF/100615.pdf)

Figure 6.2: USGS map of Lynn, MA from 1946

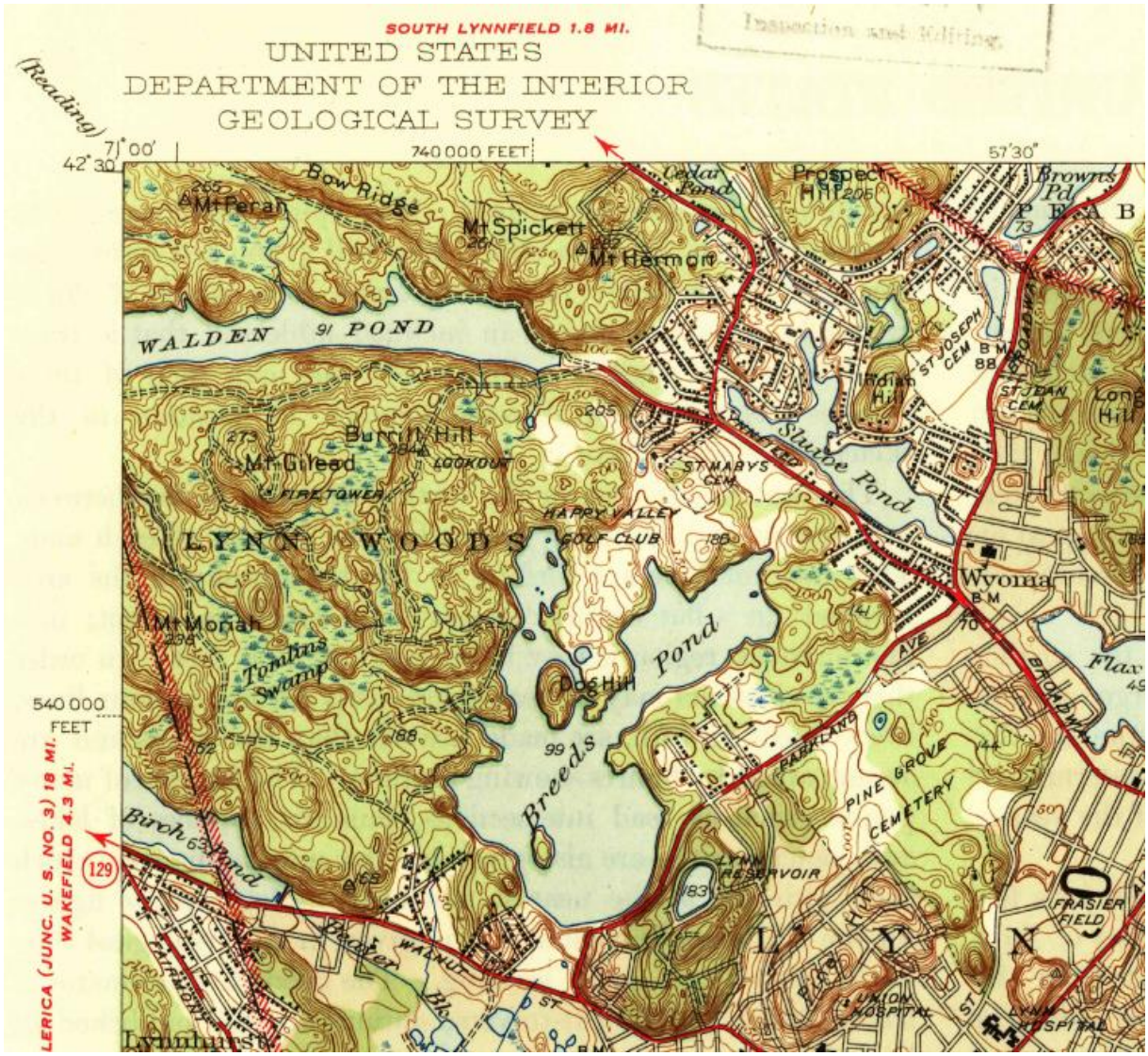


Figure 6.3: USGS map of Lynn, MA from 1970

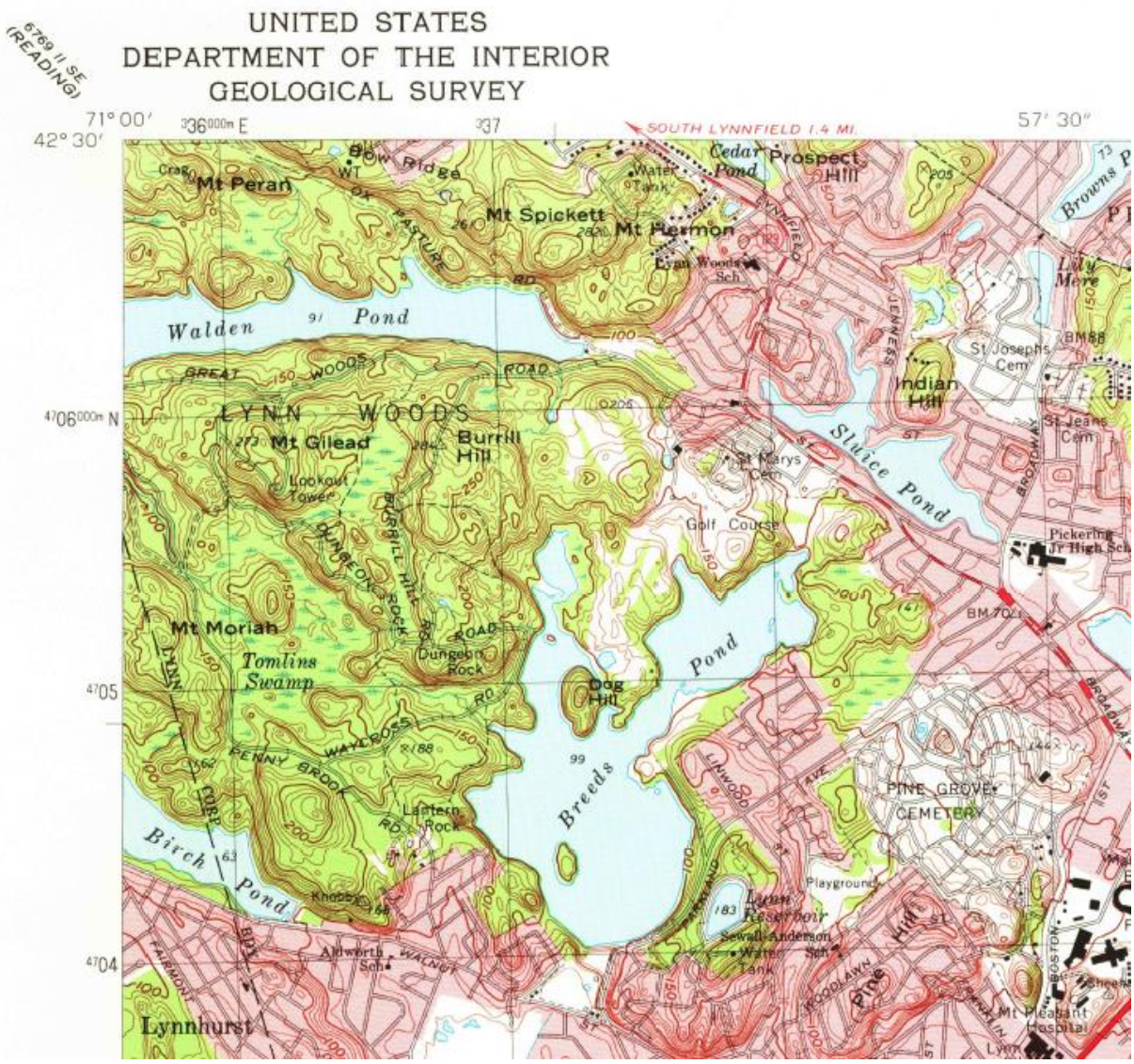
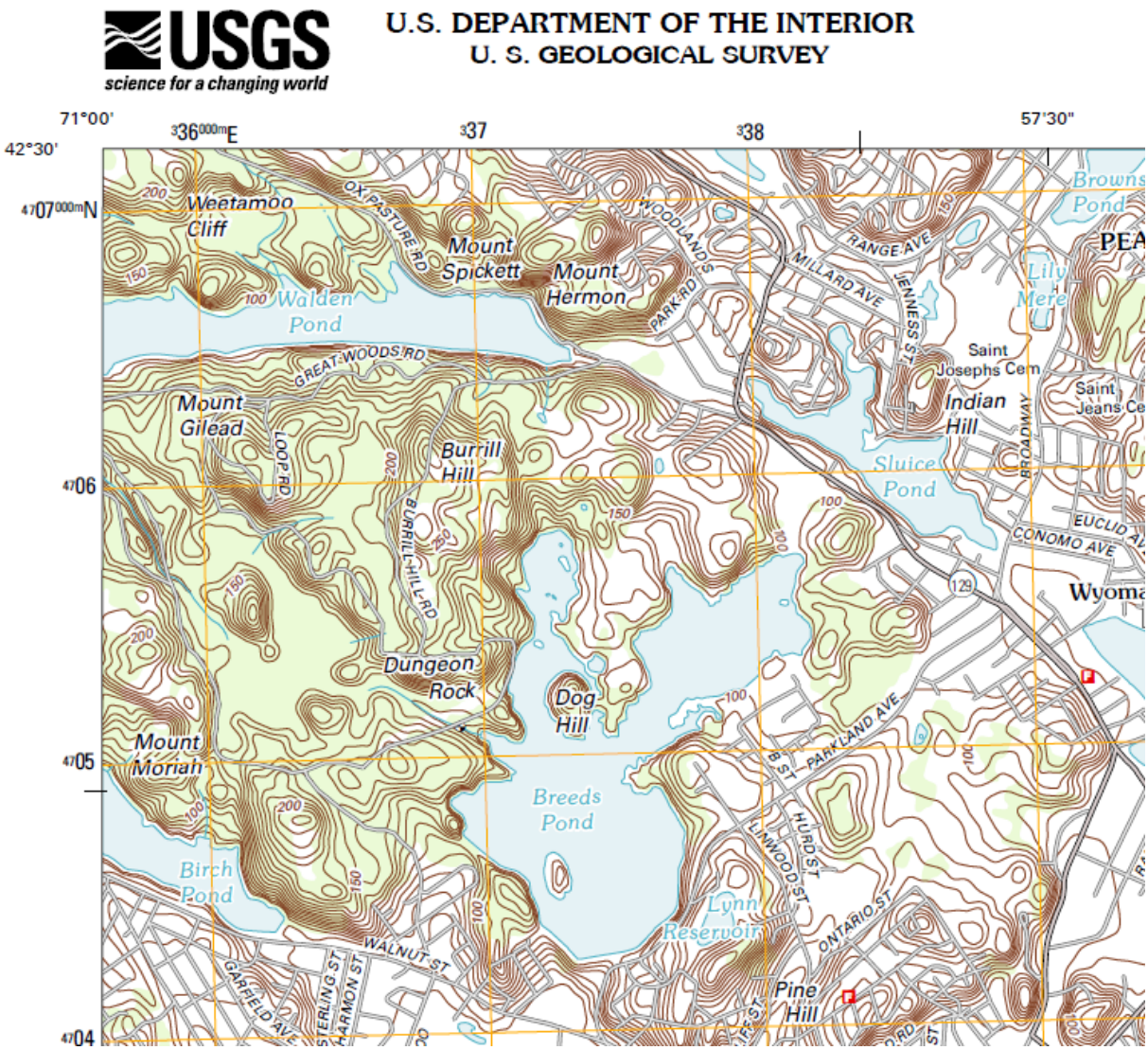
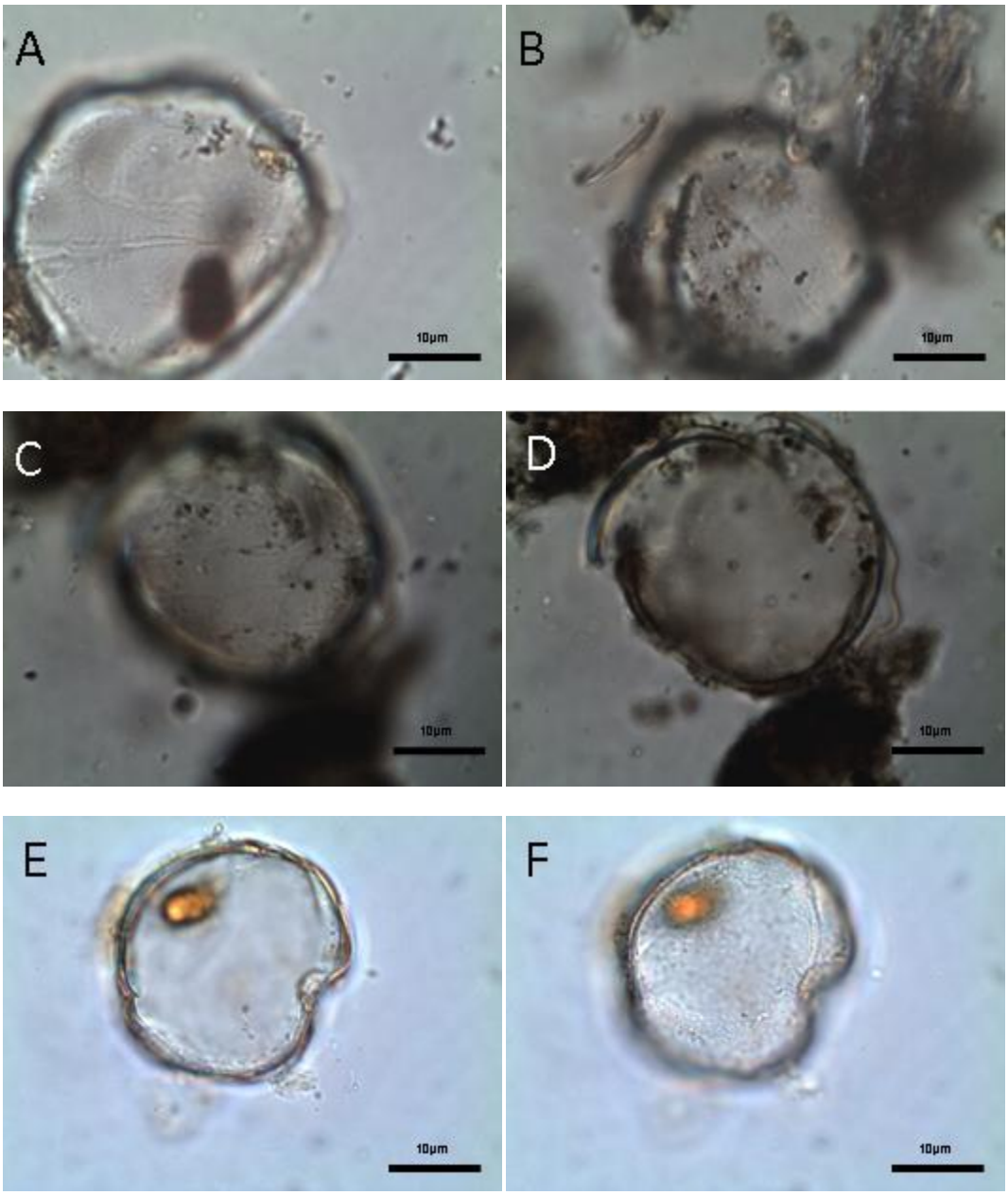
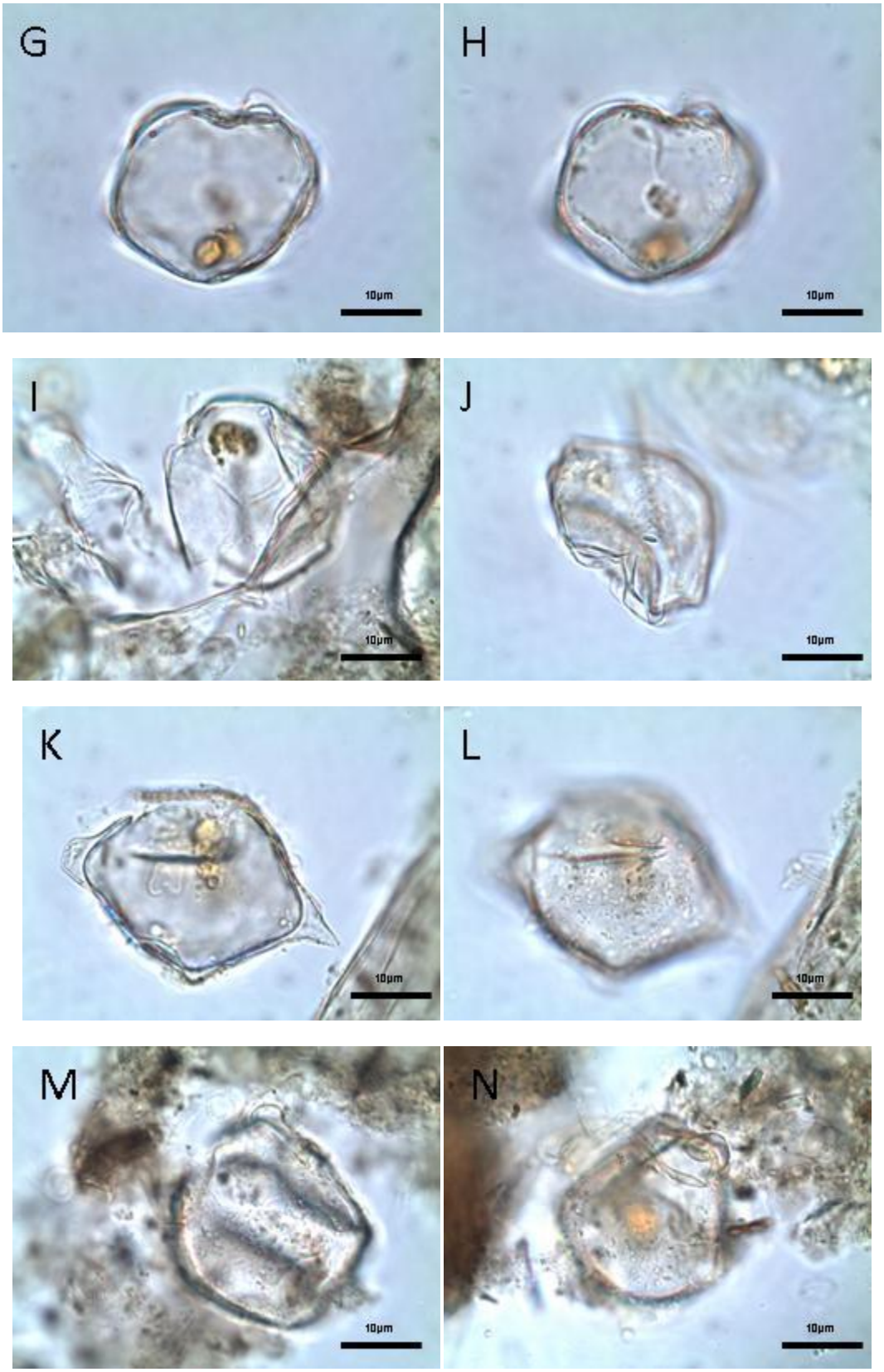


Figure 6.4: USGS map of Lynn, MA from 2012



Photoplate 6.1: Additional photographs of various dinoflagellate cysts




G
H
I
J
K
L
M
N
10µm

A-D) *Peridinium* cf. *gatunense*. Cells are spherical (36 - 50 x 40 - 47 µm) with the epi- and hyposome equal in size. Cells display a deep cingulum that is displaced approximately one cingluar width. Thecal plates are reticulated. The 1' plate is asymmetrical, 4-sided and small. Plate formula: 4', 3a, 7'', 5c, ?s, 5''', 2'''' (Hansen and Flaim 2007). Cysts are described as oval (25 - 40 x 35 - 55 µm), with 1-3 bright, red bodies, an epicystal archeopyle and 2 - 3 µm thick, smooth walls (Mertens et al. 2012). Cysts in this study large (longitudinal length: 40 – 45 µm), spherical to ovoid, cavate (possibly stoibocavate), proximate cysts. Cysts often display clear pentabular paratabulation (holotabulate) and a wide (5 µm) paracingulum. Striations perpendicular to the paracingulum are distinct on many samples. Sample: SP Core 3 0 cm V28/1; SP Core 3 (4) 0 cm G31/, I 23/1. E-F) *Peridinium willei* displaying distinct "shoulders" around the sulcus. Cells are large (42 - 63 µm x 42 - 59 µm), with the episome slightly larger than the hysosome. The cingulum is submedian and displaced one cingular width with a narrow sulcus. The thecal plates are reticulated. The 1' is large and wide. Plate formula: 4', 3a, 7'', 5c, ?s, 5''', 2'''' (Hansen and Flaim 2007). Cysts are large (48 - 58 µm x 49 - 52 µm), rounded, cavate, and proximate. The inner layer is transparent, smooth and ellipsoidal while the outer layer is slightly invaginated in the sulcal area and forms two distinct shoulders (Krueger 2012; Volik 2014). The archeopyle is transapical (McCarthy et al. 2011). Sample: SP09 S1 40 cm (beta) V48/0. G-J) *Peridinium volzii* displaying distinct "shoulders" similar to that of *P. willei.* Images display increasing degradation of *P.volzii* cysts indicating that size of cysts is an important aspect of identification. Cells are medium-sized (33 - 43 x 30 – 40 µm), oval-shaped and slightly dorso-ventrally flattened with the cingulum median or sub-median slightly displaced. The sulcus is narrow, with the sa-plate extending into the episome. Thecal plates are reticulated. The 1' plate is elongated, small and rhomboid in shape. The 4" plate is large, with the 2a plate bordering it at its anterior margin. Plate formula: 4', 3a, 7'', 5c, ?s, 5''', 2''''

(Hansen and Flaim 2007). Cysts are medium-sized (~38 - 45 µm x ~42 - 50 µm), cavate, proximate cyst, lacking ornamentation and similar to *Peridinium willei* Huitfeld-Kaas, however smaller in size (Krueger 2012). Samples: SP09 S1 40 cm (beta) V46/1; SP09 S4 74.5 cm V36/0, M39/0. K-N)*Peridinium wisconsinense* in various forms of degradation showing that apical and antapical processes are often not visible. Cells are described by Popovsky and Pfeister (1990) as spindle shaped cells with an asymmetrical plate pattern forming a plate formula that is 4′, 3a, 7″, 5‴, 2‴″) (McCarthy et al. 2011).1a plate is characteristically pentagonal with a blunt apical horn.1' plate is elongated from the cingulum to the apex pore plate (McCarthy et al. 2011). Cysts of *P. wisconsinense* are easily identified from the rounded cysts of *Peridinium volzii* Lemmerman and *Peridinium willei* Huitfeld-Kaas by its sometimes bifurcated apical horn and single, sharply pointed antapical horn (63 x 50 µm). The cysts are cavate, proximate with a smooth inner layer and an ornamented outer layer. When visible, the archeopyle encloses the operculum, and consists of apical plates 2', 3', 4' and part of the first apical plate (McCarthy et al. 2011; Volik 2014). The outer later often displays ornamentation which can be concentrated along parasutures and most pronounced at the cingulum (Mertens et al. 2012). Ornamentation appears finely to roughly sacbrate (Mertens et al. 2012). Samples: SP09 S4 74.5 cm P28/0, P36/4; SP09 S5 14 cm EF: W30/0.